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RELATION OF LYMPHOCYTIC INFILTRATION OF INFLAMMATORY ORIGIN TO ANGIOGENESIS

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IN A RECENT study[1] on the pathogenesis of Laennec's cirrhosis, morphologic evidence was adduced which showed that the mature lesions of Laennec's cirrhosis passed through the following stages: (1) the fatty liver; (2) an inflammatory cell infiltration of the periportal spaces and of the areas around the intrahepatic branches of the hepatic veins, in which the cells were mostly of the lymphoid type (lymphocytes and monocytes), associated with a smaller number of plasma cells and a few polymorphonuclears; (3) a fibroblastic differentiation of these lymphoid cells; (4) the development within these infiltrations of newly produced capillaries which formed an extensive vascular communication between the portal spaces and the hepatic veins, converting the liver into a modified Eck fistula. Following the laws of Thoma, some of these capillaries disappeared, while others persisted and matured, becoming the venous channels that coursed throughout the new connective tissue septums. In other words, the formation of new connective tissue was not considered the primary event in Laennec's cirrhosis but was viewed as arising *pari passu* with extensive angiogenesis.

The validity of this interpretation depends on the origin and the nature of this inflammatory monocytic infiltration and its potentialities for differentiation, especially in respect to angiogenesis. It is essential to emphasize at this point that I do not refer to the noninflammatory monocytic infiltrations that occur in leukemia. Leukemic infiltrations are differentiated from the inflammatory variety in that the cells stain positively with the oxidase reaction and that there is no admixture of plasma or polymorphonuclear cells.

The origin of the monocytes of inflammatory infiltrations has long been controversial. There have been four contentions: (1) that they are lymphocytes emigrating from the blood[2]; (2) that they arise by

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1. Moschcowitz, E.: Arch. Path. **45**:187, 1948.

2. (*a*) Globus, J., and Michels, N. A.: Arch. Path. **8**:371, 1929. (*b*) Jaffé, R.: The Pathology of Pulmonary Tuberculosis, in Goldberg, B.: Clinical Tuberculosis, Philadelphia, F. A. Davis Company, 1941. (*c*) Maximow, A. A.: Arch. Path. **4**:567, 1927. (*d*) Schridde, H.: Folia haemat. **4**:285, 1907. (*e*) Marchand, F.: Verhandl. d. deutsche path. Gesellsch. **16**:5, 1913.

proliferation of the fixed indifferent connective tissue cells [3]; (3) that they are produced from vascular endothelium [4]; (4) that they are due to proliferation of preexisting (fetal) perivascular lymphocytic nodular foci.[5]

That the lymphocytes emigrate from the blood vessels under inflammatory conditions has been repeatedly shown [6] and accounts in a large measure for their localizing by preference around the smaller vessels in chronic inflammation. Whether round cells may arise from fixed connective tissue cells has not been indubitably proved. Globus and Michels,[3a] studying round cell infiltration in syphilis of the nervous system and in polioencephalitis, viewed their origin as exclusively hematogenous. There is strong evidence that the infiltrating round cells may arise from the resting wandering cells. Lippmann and Plesch [7] caused aleukocytosis in animals with thorium, and when the peritoneum was injured lymphoid cell infiltrations of the inflammatory type appeared in the injured area. They also produced inflammatory mononuclear cell exudates in the cornea, which is normally avascular. Busse [8] found that in tissue cultures round cells may be derived from connective tissue cells and that they may again revert to their original state. Maximow [9] cultivated animal tissue and could trace large round cells and polyblasts from histiocytes as well as from hematogenous small and large lymphocytes. The current view is that both hematogenous cells and histiocytes are sources of these infiltrations.

That the round cell of inflammatory origin may be derived from vascular endothelium has never been decisively proved. Marchand [3a] was a staunch advocate of the specificity of endothelium and regarded the fixed connective tissue cell and the endothelial cell as independent. Foot,[10] who at first held that the round cells are directly derived from endothelium, later abandoned his view. However, I deem such a transition as highly probable but as taking place only indirectly.

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3. (*a*) MacCallum, W. G.: Text Book of Pathology, ed. 7, Philadelphia, W. B. Saunders Company, 1940. (*b*) Hueck, W.: Beitr. z. path. Anat. **66**:330, 1920. Marchand.[3a]

4. Foot, N.: J. M. Research **35**:353, 1919.

5. Ribbert, H.: Virchows Arch. f. path. Anat. **150**:391, 1897.

6. (*a*) Alzheimer, A.: Histologische Studien zur differential Diagnose der progressiven Paralyse, in Histologische und histopathologische Arbeiten über die Grosshirnrinde, Jena, G. Fisher, 1904, pp. 1 and 18. (*b*) Nissl, F.: Zur Histopathologie der paralytischen Rinderkrankung in histologische und histopathologische Arbeiten über die Grosshirnrinde, ibid., 1904, vol. 1, p. 315. Globus and Michels.[3a]

7. Lippmann, H., and Plesch, J.: Arch. f. klin. Med. **118**:281, 1915.

8. Busse, O.: Virchows Arch. f. path. Anat. **229**:1, 1920.

9. Maximow, A. A.: Physiol. Rev. **4**:533, 1924.

10. Foot, N.: J. Exper. Med. **34**:625, 1921.

As I shall later try to show, the reverse procedure is the rule both in the embryo and in chronic inflammation, namely, that the round cell may eventually be transformed into vascular endothelium. In my study of the formation of connective tissue in Laennec's cirrhosis I noted strong evidence of widespread obliteration of the newly formed capillaries, the remnants of which assume irregular branching forms and revert to cells of the mesenchymal type. Others [11] have observed this phenomenon.

The final suggestion, namely, that some of these round cell infiltrations arise from hyperplasia of preexisting (fetal) perivascular lymphatic

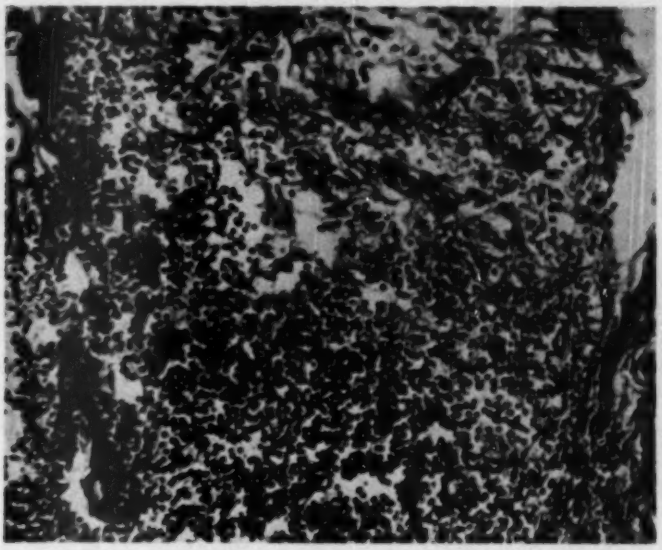

Fig. 1.—Lymphocytic focus in aorta showing intact area (left) and area (right) undergoing fibroblastic differentiation and capillarization.

foci, was made by Ribbert.[8] These are of microscopic dimensions. He called such foci "lymphadenoid granulation tissue." They have a special tendency to localize around capillaries or small arterioles. They resemble lymph nodes, but they differ in that they are not sharply defined and/or

11. (*a*) Rössle, R.: Die Entzündungen der Leber, in Henke, F., and Lubarsch, O.: Handbuch der speziellen Pathologie und Histologie, Berlin, Julius Springer, 1930, vol. 5, pt. 1. (*b*) Benninghof, A., in von Mollendorf, W.: Handbuch der mikroskopischen Anatomie des Menschen, ibid., 1930. (*c*) Bailey, P., and Miller, A. M.: Text Book of Embryology, ed. 5, Baltimore, William Wood & Company, 1929. (*d*) Werthmann, A.: Virchows Arch. f. path. Anat. **270**:605, 1928.

possess no capsule, and lack the delicate argyrophil reticulum between the cells.[12] They bear an unusually close resemblance to the lymphatic deposits normally found in the lungs along the finer tributaries of the bronchi and the pulmonary vessels, but they are present in chronic inflammation of organs in which such lymphatic foci normally are absent—for instance, the kidney, the thyroid gland, the liver and the heart. For this reason it is difficult to accept Ribbert's interpretation of their origin. On closer inspection of these foci one notes in many of them an admixture of plasma cells and in some fibroblastic transformation with newly formed capillaries precisely like those observed in granulation tissue (fig. 1). I believe, therefore, that they represent perivascular aggregates of inflammatory cells more closely packed than usual.

EVOLUTION OF MONONUCLEAR (ROUND CELL) INFLAMMATORY CELLS

Despite the confusing nomenclature that has been imposed on primitive and adult mesenchyme it has been amply demonstrated by morphologic evidence, by tissue culture[13] and by the transparent chamber made in the living animal[14] that the lymphocyte, the plasma cell, the clasmatocyte, the monocyte, the wandering cell, the fibroblast, etc, are not independent and unrelated individualities but represent various phases of development of the mesenchymal cell under the influence of various stimuli.[15] Indeed, in studying chronic inflammatory lesions from the earliest to the later phases one usually finds such cells in variable quantities. In the early phases the cells are mostly of the mononuclear and of the lymphoid type. In the later phases they become plasma cells, macrophages, clasmatocytes, etc. Ultimately fibroblasts become dominant.

CONDITIONS UNDER WHICH THESE DERIVATIVE CELLS DEVELOP TO THE POINT OF ANGIOGENESIS

In the previous study [1] I observed that in the fibroblastic phase there was a new formation of capillaries. That these were not previously existing capillaries was evidenced by their large numbers in situations

12. Hellmann, T.: Lymphgefässe, Lymphknötchen, in von Mollendorf, Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol. 6, pt. 1, p. 233.

13. Maximow, A. A.: Proc. Soc. Exper. Biol. & Med. **24**:570, 1927.

14. Clark, E. R.; Hitschler, W. J.; Kirby-Smith, H. T.; Rex, R. O., and Smith, J. H.: Anat. Rec. **60**:129, 1931.

15. Adami, J. G.: Principles of Pathology, ed. 2, Philadelphia, Lea & Febiger, 1910, vol. 1. Globus and Michels.[9a] Marchand.[9c] Foot.[10] Benninghoff.[11b] Werthmann.[11d] Hellmann.[12] Maximow.[13]

where normally they are sparse or nonexistent, by their delicate walls and by the prominent endothelium. That they were not sinusoids was evidenced by the absence of Kupffer cells and by their coursing in every direction. The observation that such newly formed capillaries never appeared until the fibroblastic differentiation had been sufficiently advanced suggested the possibility that the formation of these capillaries was of autochthonous origin. This implies that vascular endothelium is one of the end products of the progressive differentiation of these inflammatory cells and its production represents part of the reparative process inherent in the concept of inflammation. Curiously, this mechanism was suggested by Billroth [16] in 1856 and later by Ranvier,[17] both of whom noted that the mechanism of the production of these capillaries bore a remarkable similarity to that which occurs in the embryo. Ranvier claimed that the new capillaries were formed through the intervention of certain cells, the *cellules vasoformatives,* but these cells are not specific entities, since they represent only cells that are approaching the later stages of fibroblastic differentiation. Subsequently the autochthonous mechanism was relegated to the background, owing to the wide acceptance of the angioblast theory of His,[18] which predicates that endothelium arises from vessels which first appear in the yolk sac of the embryo and that from these are developed the vessels of the body proper. Years later, Rückert and Mollier [19] revived the autochthonous mechanism of angiogenesis.

Embryologists accept the fact that in the early embryo blood islands appear which are formed by differentiation of the mesenchymal cells of the area pellucida. Eventually the outer layer forms a space which is lined by endothelium and becomes the primitive blood vessel. The remaining cells are converted into hemocytoblasts, the progenitors of the blood cells. These primitive spaces arise from numerous islands and form a plexus of capillaries which later fuse to form the main vascular trunk. The decisive refutation of the angioblastic theory and therefore of the assumption that endothelium springs only from endothelium and from no other source was the observation made by Miller and McWhorter,[20] who cut away the lateral half of the entire area opaca of the blastoderm in the developing chick embryo at a time when no blood vessels had appeared in the area pellucida: In four to forty-eight

16. Billroth, T.: Die Allgemeine chirurgische Pathologie und Therapie, ed. 13, Berlin, G. Reimer, 1887, p. 69.

17. Ranvier, L. A.: Arch. d. anat. microbiol. **3**:122, 1900.

18. His, W.: Anatomie menschlichen Embryonen, Leipzig, F. C. W. Vogel, 1880.

19. Rückert, J., and Mollier, S., in Hertwig, W. A. O.: Handbuch der vergleichenden und experimentelle Entwicklungslehre der Wirbeltiere, Jena, Gustav Fischer, 1906, vol. 1, pt. 1, p. 126.

20. McWhorter, J. E., and Miller, A. M.: Anat. Rec. **8**:203, 1914.

hours blood vessels invariably appeared on the injured side as well as on the uninjured side. This experiment was confirmed by Reagan [21] and Stockard.[22] The subsequent development of the vascular system proceeds according to the laws of Thoma.[23] These are:

1. The width of a vessel is dependent on the rate of the blood flow: When the rate lessens, the width diminishes.

2. The thickness of the wall depends on the blood pressure.

3. The new formation of capillaries depends on the difference of pressure between the interior of the capillary and the surrounding tissue spaces. If in a certain area the pressure of the fluid is higher than the intracapillary pressure, the formation of capillaries does not occur. If, on the other hand the intracapillary pressure is higher, new capillaries are engendered.

These laws have withstood rigid tests and are generally accepted by morphologists. It is also recognized that the portion of the primitive capillary network that persists precedes the formation of the large vessels. Bailey and Miller [11c] stated that "the vitelline plexus of capillaries antedates any of the large vitelline vessels." According to the first and second laws of Thoma, the vessel wall elongates and thickens, and, by the conversion of the surrounding mesenchymal cells (pericytes), fibrillar connective tissue and elastic and muscular coats are formed, and the vascular bed develops into the mature vascular system. This concept of the mechanism is now widely held by pathologists in respect to new blood vessels forming in granulation tissue.[24] The laws of Thoma apply to vessels forming in granulation tissue as well as to those forming in the embryo.[24e] The one difference between embryonal and inflammatory angiogenesis consists in the circumstance that in the embryo the stimulus is that of growth, while in inflammation it is that of repair. Long ago [25] I made the independent observation that in the genesis of ossification of calcified corpora lutea of the ovary angiogenesis was produced by an arrangement of fibroblasts along the calcified margin forming a circular channel with eventual flattening of the bordering cells into an endothelial lining. This is precisely the description given by Billroth,[1b] Werthmann,[11d] Hueck [9b] and Thin [24b] in their descriptions of the mechanism of angiogenesis as observed in granulation tissue. In other words, in chronic inflammation newly elaborated mesenchyme acquires

21. Reagan, F. P.: Anat. Rec. **9**:329, 1915.

22. Stockard, C. R.: Am. J. Anat. **18**:227, 1918.

23. Thoma, R.: Beitr. z. path. Anat. **66**:378, 1920.

24. (*a*) Dawson, J. W., cited by Beattie, J. M., and Dickson, W. E.: A Text Book of Special Pathology, ed. 3, St. Louis, C. V. Mosby Company, 1927 (*b*) Thin, G.: Quart. J. Micr. Sc. **16**:241, 1876. (*c*) McClure, C. F.: Anat. Rec. **23**:219, 1921. (*d*) Borst, M.: Ergebn. d. path. Anat. **4**:461, 1897. (*e*) Sandison, J. C.: Am. J. Anat. **41**:470, 1928. (*f*) Miniverni, R.: Virchows Arch. f. path. Anat. **204**:75, 1911. (*g*) Hueck.[9b] (*h*) Werthmann.[11d]

25. Moschcowitz, E.: Johns Hopkins Hosp. Bull. **27**:1, 1916.

the pluripotential characters of its embryonal predecessor. Even extramedullary blood formation is sometimes produced in chronic inflammation, but only in tissues that possessed blood-forming properties in embryonal life, notably the spleen. It is not infrequent in congestive splenomegaly of long standing. Jordan [26] called it a phylogenetic reversion.

There is no doubt that in the later phases of chronic granuloma new vessels may also be produced by sprouting. There is abundant testimony that this occurs.[27] Clark [28] actually observed it in the transparent chamber inserted into the rabbit's ear. I [1] have described capillaries that penetrated directly into the hepatic lobule from venules of the newly formed perilobular granulation tissue of Laennec's cirrhosis which were entirely unaccompanied by any cellular proliferation.

While I feel that a larger part of the new connective tissue in Laennec cirrhosis is the result of the obliteration of capillaries that did not survive, there is every evidence that a considerable moiety is due to direct fibroblastic differentiation without the intermediacy of angiogenesis. Such a function has long been accorded to mesenchyme. Indeed, as I shall point out, angiogenesis is not nearly as prominent in other chronic granulomas, and the healing process represented by fibrosis is largely instituted by the laying down of collagen fibers under the influence of fibroblasts.

With the validity of autochthonous angiogenesis as a premise, it seemed pertinent to determine whether an identical mechanism is present in various types of chronic inflammation of other organs. As the criterion of chronic inflammation I refer to a localized collection of round cells, most of which are undoubtedly lymphocytes derived from blood vessels by emigration, with an admixture of a greater or lesser number of plasma cells and sometimes a few polymorphonuclear leukocytes. Various stages of the process were observed, from the earliest infiltrative phase to the ultimate, with sclerosis.

It must be emphasized that angiogenesis is not observed in every chronic inflammatory focus. It is found only when the fibroblastic differentiation has attained a fairly advanced stage. Nor is it observed in the terminal phases of the healing process, when the stage of obliteration of the newly formed capillaries and sclerosis has occurred.

The causes of inflammatory round cell infiltration are various. It often follows an acute suppurative bacterial infection (chronic thyroid-

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26. Jordan, H. E.: Arch. Path. **18**:1, 1934.

27. Yamigawa, K.: Virchows Arch. f. path. Anat. **132**:446, 1893. Meyer, J.: Ann. des Charité Krankenhaus, Berlin, **4**:41, 1853. Kölliker, A.: Entwickelungsgeschichte des Menschen und der höheren Tiere, Leipzig, William Engelmann, 1879. Marchand.[2e] Borst.[24d] Werthmann.[11d]

28. Clark, E. R.: Am. J. Anat. **23**:37, 1918.

itis, chronic pyelonephritis and chronic mastitis); presence of a known infectious agent that predisposes to round cell proliferation (tuberculosis, syphilis); a virus infection (infectious hepatitis); an infection of unknown origin (rheumatic fever, chronic thyroiditis, chronic mastitis, chronic pancreatitis); foreign body reactions (silicosis); undernutrition combined with an absence of lipotropic factors (Laennec cirrhosis); obstruction of the bile duct with or without infection (biliary cirrhosis); thrombosis; diseases of obscure origin (peptic ulcer, nonspecific ulcerative colitis, terminal ileitis); glomerulonephritis and venous hypertension (congestive splenomegaly). The granuloma that sometimes surrounds a carcinoma and Hodgkin's disease have also been included.

Necrosis has a direct influence on the amount and the intensity of the formation of the granuloma, as exemplified in tuberculosis, in gummas and in periarteritis nodosa, but it is not essential. Necrosis is usually attended with the emigration of polymorphonuclear leukocytes.

As a rule, angiogenesis is more pronounced if the chronic granuloma follows an acute process.

Tuberculosis.—In early experimental tubercles, especially those with virulent organisms, the predominant cells are the polymorphonuclear leukocytes. These later give way to monocytes. The amount of mononuclear cell infiltration around tubercles depends on a large number of factors: the dosage of bacilli, their virulence and the susceptibility or the resistance of the animal. According to Rich,[29] the lower the resistance and the higher the virulence the larger will be the number of mononuclear cells. Rich makes a distinction between the lymphocytes and the mononuclear cells, but the foregoing exposition, I believe, makes this doubtful. Indeed, Maximow,[30] in cultivating tuberculous mammalian tissue in vitro, traced the transition of the lymphocyte to the monocyte to the plasma cell and the polyblast and reversely. These cells partake in the formation of the epithelioid cells, and the latter, by fusion, form the Langhans giant cell. Conventionally it is stated that the tubercle is avascular, but in the study of a large number of tubercles I have found in the fibroblastic periphery newly formed capillaries (fig. 2). These are sparser than in other forms of chronic inflammation. Jaffé [30b] confirmed this observation. Fibrosis of tubercles is therefore dependent more on direct fibroblastic activity than on angiogenesis. When a suppurative infection is associated—for instance, in a sinus or in cavity formation—angiogenesis is more conspicuous.

Syphilis.—The characteristic infiltration of syphilis consists of mononuclear cells of the lymphocytic type with a considerable admixture of plasma cells. I have observed a number of syphilitic lesions (hepar

29. Rich, A. R.: The Pathogenesis of Tuberculosis, Springfield, Ill., Charles C Thomas, Publisher, 1944.

30. Maximow, A. A.: J. Infect. Dis. **34**:549, 1924.

lobatum, congenital syphilis of the lungs, testis and pancreas, aortitis, gumma of the trachea) in the fibroblastic phase and find in these areas fairly active angiogenesis, certainly greater than in tuberculosis (fig. 3). The tendency of the syphilitic infiltrate to capillarize has been remarked by a number of observers.[31]

Rheumatic Fever.—Gross and his co-worker Friedberg [32] described leaflets of cardiac valves in which there were infiltrates consisting of lymphocytes and plasma cells and occasional polymorphonuclear leukocytes; eventually these underwent fibroblastic differentiation of a granulomatous nature and capillarization. Although they do not ascribe any

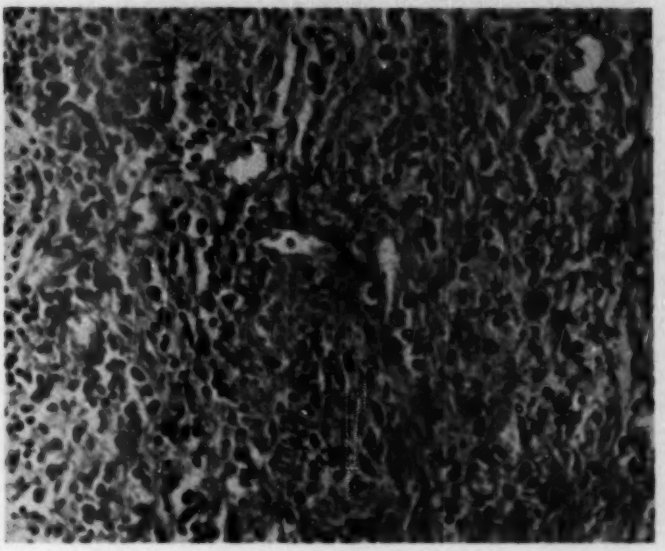

Fig. 2.—Pulmonary tuberculosis: capillarization in infiltrate around tubercle.

mechanism to the capillarization, there is no doubt from my own study that it is autochthonous. Gross contended that the valves of the heart are normally avascular and that when capillarization is observed it is an evidence of a previous rheumatic infection. I have observed a considerable number of vascularized valves, and the vascularizations

31. Boyd, W.: A Text Book of Pathology, ed. 4, Philadelphia, Lea & Febiger. 1943. Fukushi, M.: Virchows Arch. f. path. Anat. **211**:331, 1913. Globus and Michels.[28] Alzheimer.[66]

32. Friedberg, C., and Gross, L.: Am. J. Path. **12**:183, 1936. Gross, L., and Friedberg, C.: ibid. **12**:855, 1936. Gross, L.: ibid. **13**:275, 1937.

are always accompanied by marked fibroblastic change (fig. 4). I have also noted similar processes in the pericardium.

There is another lesion of rheumatic fever, first described by Klotz [33] and later elaborated by Von Glahn and Pappenheimer,[34] consisting of lymphocytic and plasma cell infiltrations around the vasa vasorum of the aorta and its larger branches with eventual fibroblastic change and capillarization. I have not had the opportunity to study such a case.

Toxic Hepatitis and Acute Yellow Atrophy.—I have linked these two maladies together because in pathogenesis they are similar. In

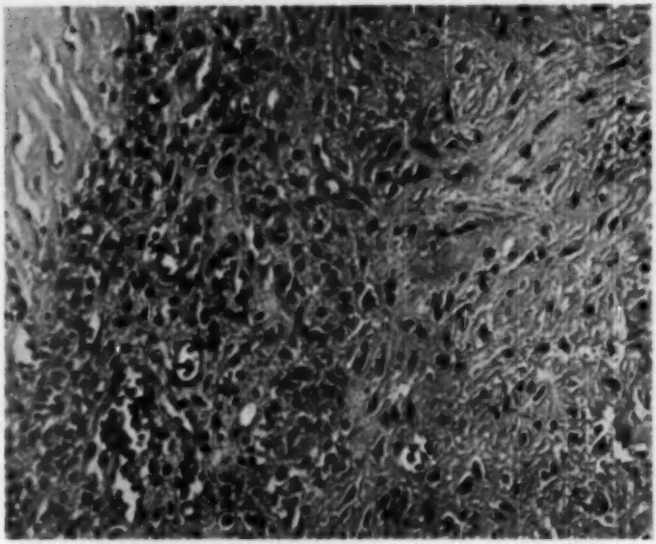

Fig. 3.—Syphilitic aortitis: capillarization in subintimal infiltrate.

the destroyed framework of subacute and chronic phases there is extensive cell reaction with mononuclear cells, plasma cells, lymphocytes, occasional neutrophilic granulocytes and, in relatively small numbers, eosinophilic granulocytes.[35] I have observed fibroblastic differentiation and capillarization in such areas.

33. Klotz, O.: Path. & Bact. **5**:18, 1913.

34. Pappenheimer, A. M., and Von Glahn, W. C.: J. M. Research **44**:489, 1924. Von Glahn, W. C., and Pappenheimer, A. M.: Am. J. Path. **2**:235, 1926.

35. Lucke, B.: Am. J. Path. **20**:471, 1944. Lucke, B., and Mallory, T.: ibid. **22**:867, 1946.

It is apparent that in these disorders the eventual fibrosis does not represent a mere condensation of fibrous stroma of the liver, as some aver, but an active proliferation.

Obstructive Biliary Cirrhosis.—That round and plasma cell infiltration of the periportal spaces occurs in obstruction of the biliary passages is a well attested observation,[36] and although it is more pronounced when there is an associated infection, it occurs without infection, as exemplified in congenital atresia and in experimental ligation of the common bile duct. In my series there have been various causes of the biliary cirrhosis: obstruction of the common duct due to stone, with and

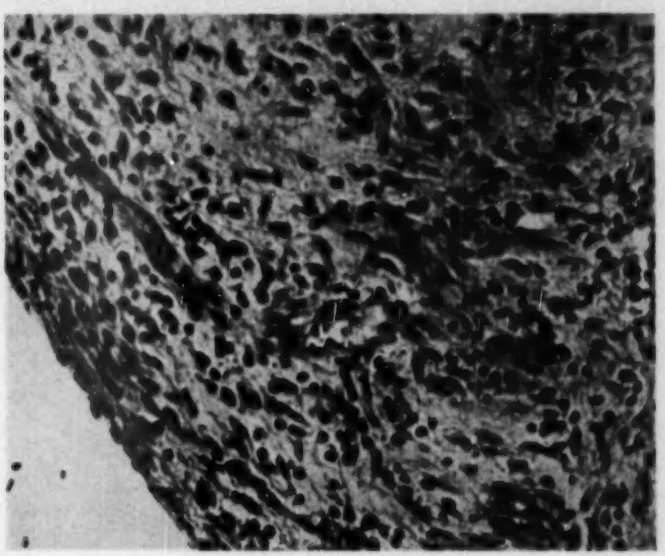

Fig. 4.—Acute rheumatic fever as found in a child 6½ years of age: capillarization in tricuspid valve, early phase.

without cholangiolitis; postoperative stricture; carcinoma of the papilla of Vater, and congenital atresia of the bile duct. In all of these, round and plasma cell infiltrations are not infrequent, especially around the finer radicles of the bile duct in the periportal spaces, with abundant new formation of bile ducts and fibroblastic differentiation and capillarization.

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36. Moore, R. A.: A Textbook of Pathology, Philadelphia, W. B. Saunders & Company, 1944. Meyenburg, H.: Virchows Arch. f. path. Anat. **221**:382, 1916. MacCallum.[5a] Rössle.[11a]

In toxic hepatitis, yellow atrophy and biliary cirrhosis, capillarization is not nearly as abundant as in Laennec cirrhosis. The ultimate fibrosis is therefore more largely dependent on direct fibroblastic change.

Chronic Pancreatitis.—This may follow an acute attack. It is often associated with cholelithiasis, obstructions of the duct of Wirsung (the pancreatic duct) and Laennec's cirrhosis. Often the cause is not discoverable. In our experience chronic pancreatitis in the fibroblastic stage is not common. I have observed 4 cases; in 3 the condition was due to obstruction of the duct of Wirsung by carcinoma of the head of the pancreas and in 1 it was associated with ulcerative colitis. All

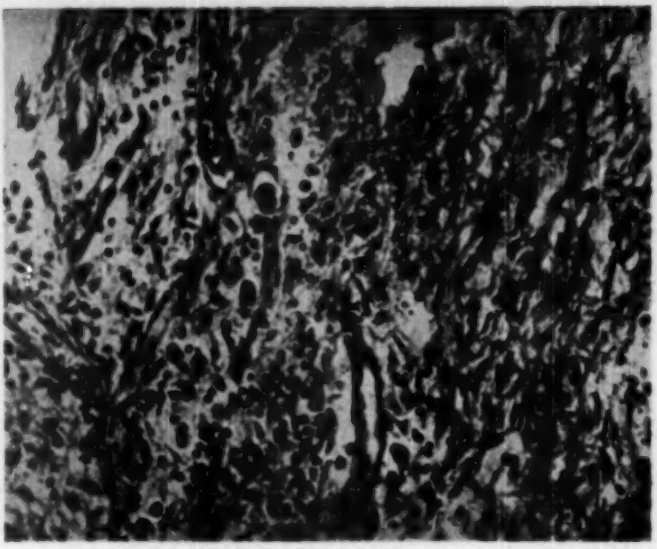

Fig. 5.—Chronic pancreatitis: capillarization in fibroblastic infiltrate.

showed areas of lymphocytic infiltration with a sprinkling of plasma cells. Areas of fibroblastic transformation and capillarization were fairly common (fig. 5).

Glomerular Nephritis.—In subacute and chronic glomerular nephritis there is often a greater or lesser infiltration of the interstitium with inflammatory cells—lymphocytes, plasma cells and occasional polymorphonuclear leukocytes. In the kidney it is sometimes difficult, owing to the abundant capillary network, to distinguish between preformed and newly formed capillaries. The latter, however, can be distinguished from the former by the fact that they have no well defined fibrous wall,

that the endothelium is high, that their topography is different and that they are always in an area of fibroblastic differentiation (fig. 6). Pure lymphocytic infiltrations are particularly abundant in these conditions, and transitions to fibroblastic differentiation and capillarization are noted.

In chronic pyleonephritis, fibroblastic differentiation and capillarization are more abundant than in glomerulonephritis, especially if associated with an acute process.

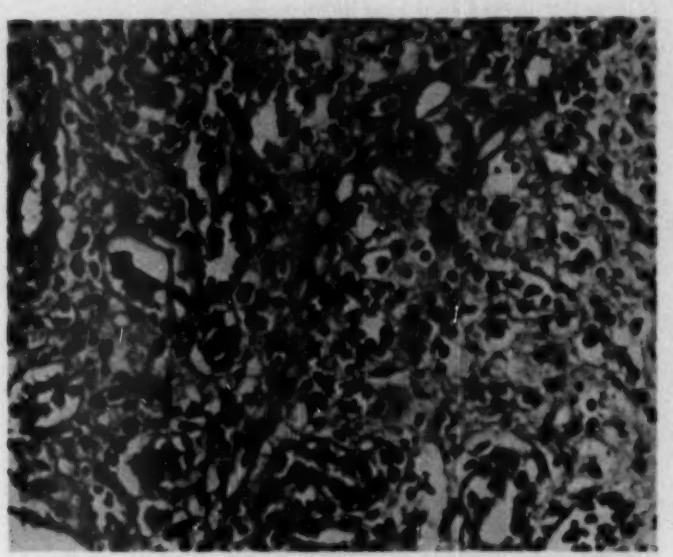

Fig. 6.—Chronic glomerulonephritis: capillarization in fibroblastic infiltrate in cortex.

In malignant nephrosclerosis the lymphocytic infiltration is not associated with plasma cells nor have I noted capillarization. The fibrosis is the result of the eventual transformation of the lymphoid cells.

Organizing Pneumonia.—The earliest phase is characterized by an exudation of mononuclear or lymphoid cells involving the alveolar spaces, accompanied by more or less fibrin. Later, plasma cells appear and fibroblastic differentiation occurs, with capillarization, which eventually proceeds to sclerosis (fig. 7). Of 4 such cases, 1 was an instance of actinomycosis, and 1 was a case of Libman-Sacks disease in which the lesion was shown in various stages; in the third the cause was unknown and various stages were observed; the fourth was a case of lipid pneu-

monia due to many years' ingestion of liquid petrolatum. It is interesting that Cornil[37] years ago described the autochthonous formation of capillaries in organizing pneumonia.

Silicosis.—Experimentally, the earliest lesion of silicosis is evidenced by lymphocytes, large mononuclear cells and plasma cells exuding into the tissues. Later, fibroblastic differentiation appears with capillarization.[38] In the lung, organizing pneumonia may arise.[39] Eventually, complete sclerosis ensues. In 2 cases we found abundant capillarization in areas that showed fibroblastic differentiation (fig. 8).

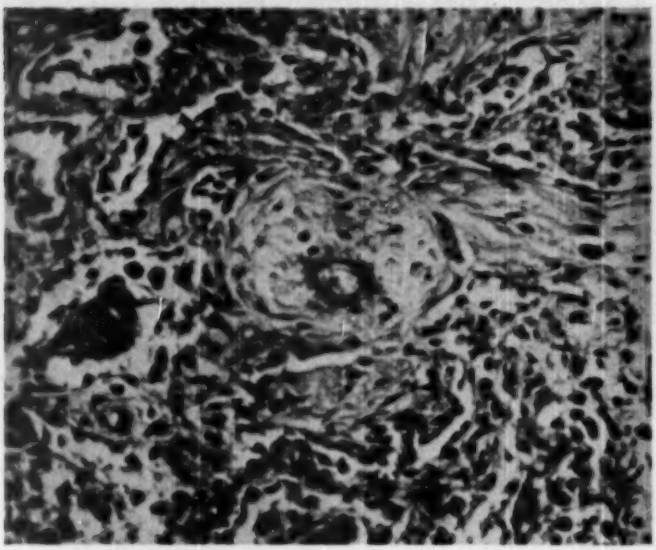

Fig. 7.—Organizing pneumonia: capillarization in alveolar exudate, late phase.

Chronic Thyroiditis.—There is doubt whether chronic thyroiditis (Riedel's disease) and struma lymphomatosa (Hashimoto's disease) are separate disorders. The first is characterized by a diffuse infiltration of round and plasma cells, while the second is represented by a diffuse infiltration of cells of the pure lymphoid type, with occasional germinal

37. Cornil, cited by Borst.[344]

38. Gye, W. E., and Kettle, E. H.: Brit. J. Exper. Path. **3**:241, 1922. Policard, A.: Presse méd. **41**:89, 1933. Schmidtman, M., and Lubarsch, O.: Handbuch der speziellen pathologische Anatomie und Histologie, Berlin, Julius Springer, 1930, vol. 32, pt. 2.

39. Cortero, I.: Am. J. Path. **24**:49, 1948.

centers. Both infiltrations are associated with a diffuse fibrosis of the gland. I have studied a considerable number of both varieties, and while I have found a few that conform to the classic description, the majority show elements of both, so that I am inclined to believe, with others [40] that pathogenetically, at least, Hashimoto's struma represents the early phase of Riedel's struma. Confirmatory of this surmise is the fact that cases have been reported in which biopsies made years apart show such a transition.[40b] In a number of instances in which the

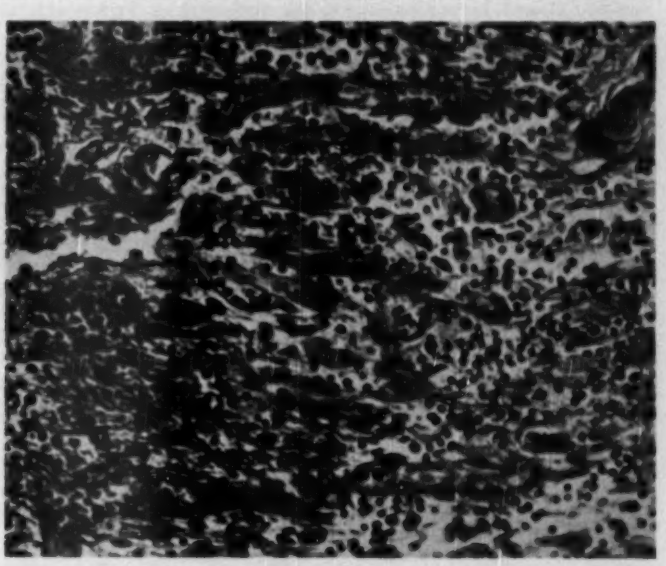

Fig. 8.—Silicosis of lung: capillarization in interalveolar infiltrate, early phase.

diagnosis was either Riedel's or Hashimoto's struma I have noted fibroblastic differentiation and capillarization.

Chronic Mastitis.—This lesion may arise with or without an acute inflammation. I have studied 6 cases, 3 in which there was an associated acute infection, characterized by an excess of polymorphonuclear leukocytes in the infiltrated areas, and 3 in which the dominant cells were round cells and a lesser number of plasma cells. In the breast, cells containing lipoid material are sometimes abundant. The infiltrations were mostly around the ducts. In all there were fibroblastic

40. (*a*) Jaffé, R. H.: J. A. M. A. **108**:105, 1937. (*b*) Joll, C. A.: Brit. J. Surg. **40**:27 and 35, 1939.

changes, with capillarization. In chronic mastitis resulting from an acute attack the capillarization is much more abundant. In long-standing cases of chronic mastitis sclerosis occurs with cyst formation.

Thrombosis.—The view is conventionally held that when a thrombus is organized this is accomplished through the sprouting of capillaries from the intima. No direct proof of such a mechanism is forthcoming. In the earliest phases, at least, I have not noted penetration of endothelium unaccompanied by fibroblastic cells. If the process of organization is followed in various stages, one finds that it begins with an infiltration of lymphoid and plasma cells usually at the base of the

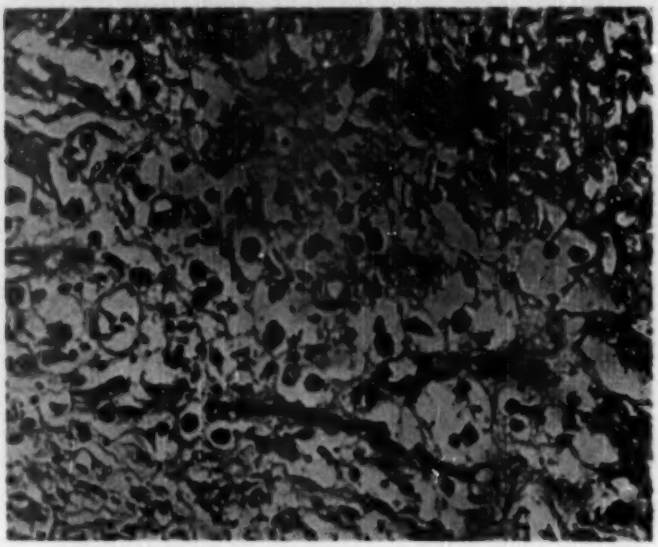

Fig. 9.—Thrombosis: capillarization in thrombus, early phase.

thrombus, where the latter is attached to the intima, with subsequent fibroblastic change and capillarization and eventually sclerosis [41] (fig. 9).

Periarteritis Nodosa.—This lesion presents different stages. The earliest is probably that described by Fishberg,[42] which appeared seven

41. Heuking, E., and Thoma, R.: Virchows Arch. f. path. Anat. **109**:288, 1887. Benda, C., in Henke, F., and Lubarsch, O.: Handbuch der speziellen Pathologie und Histologie, Berlin, Julius Springer, 1930. Brown, G. E.; Allen, E. V., and Mahorner, H. R.: Thromboangeitis Obliterans, Philadelphia, W. B. Saunders Company, 1928.

42. Fishberg, A. M.: Virchows Arch. f. path. Anat. **240**:484, 1922.

days after the onset of the illness. There was endothelial proliferation of the intima. The media and the adventitia were infiltrated with fibrin, polymorphonuclear leukocytes, a few lymphocytes and eosinophilic granulocytes. Later, cells of the lymphoid type predominate, with a lesser number of plasma cells. These infiltrates subsequently undergo fibroblastic transformation and eventually capillarization (fig. 10). The accompanying necrosis of the vessel wall undoubtedly contributes to the unusually abundant, excessive vascularization. I have studied 9 cases in which various stages of the process were represented. In these

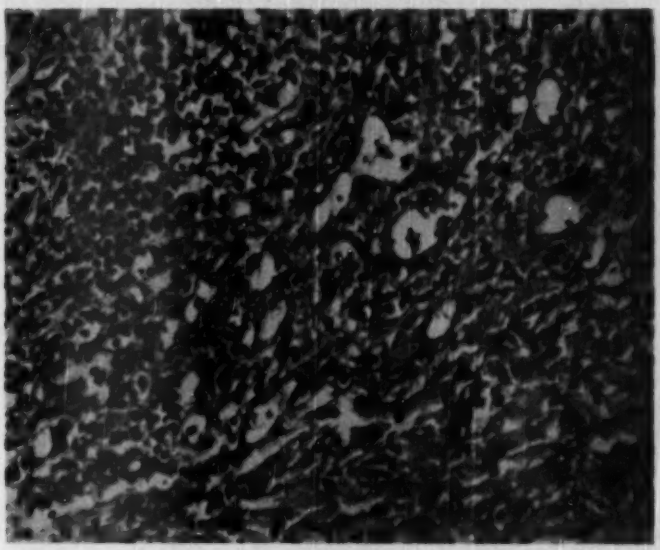

Fig. 10.—Periarteritis nodosa: capillarization in infiltrate, late phase.

cases the following organs were involved: stomach, intestine, pancreas, gallbladder, adrenal gland, striated muscle, liver and prostate.

Venereal Lymphogranuloma.—The stages of the primary lesion were studied by Sheldon and Heyman.[43] At first, there is an infiltration of large mononuclear and plasma cells which arise from the adventitia of the small blood vessels near the perivascular lymphatic space. Later, fibroblastic proliferation ensues, with new vessels being formed at the periphery of the infiltration. I have had the opportunity to study 2 cases in which capillarization was evident.

43. Sheldon, W. H., and Heyman, A.: Am. J. Path. **23**2:653, 1947.

Neoplasms.—Cancers vary in the intensity of mononuclear cell infiltration in their periphery. As a rule, those that show the greatest morphologic activity show fewer round cells than the less active growths. Cellular proliferation may even be completely absent. Sometimes the primary tissue is infiltrated while the metastases are free, and/or vice versa. The cells are mostly of the lymphoid type. Occasionally one notes plasma cells and not infrequently polymorphonuclear leukocytes. The latter are, as a rule, associated with necrosis. Ewing [44] and Ribbert [45] described a granulation tissue with capillarization in certain phases. In fact, Ribbert expressed the belief that in epithelioma the granulation

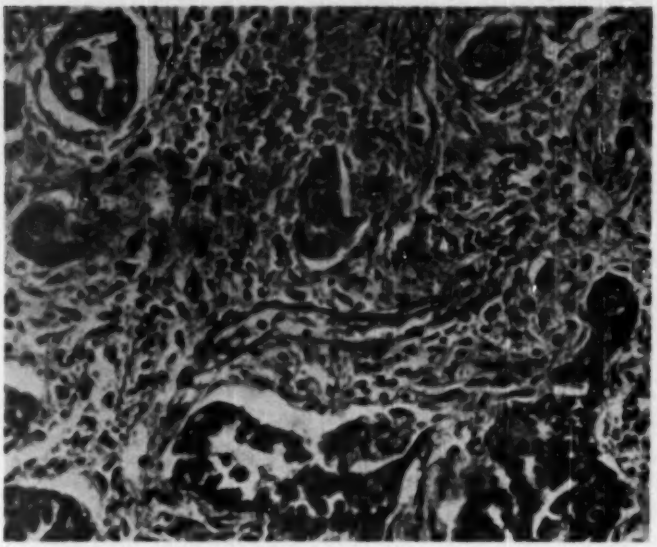

Fig. 11.—Carcinoma of esophagus: capillarization in an area of lymphocytic infiltration.

tissue is a primary event and that, by isolating some of the epithelial cells, it stimulates the activity which progresses into cancer. Both Ewing and Murphy [46] viewed these lymphoid cell infiltrations as a defensive process. They are a noteworthy feature in experimental grafting of heteroplastic tissue. Sampson [47] described a number of

44. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940.

45. Ribbert, H.: Virchows Arch. f. path. Anat. **134**:433, 1894.

46. Murphy, J. B.: J. A. M. A. **62**:1459, 1914.

47. Sampson, J. A.: Am. J. Path. **7**:423, 1931.

instances in which the implanting in human subjects of the peritoneum of cells of an adenocarcinoma of the ovary caused a well defined encircling area of granulation tissue.

In the examination of many neoplasms I collected 5 that showed well defined granulomas along the periphery; the organs involved were: breast, lung, esophagus, stomach and colon, with a metastasis involving the colon (figs. 11 and 12).

"Congestive" Splenomegaly.—In a recent communication [48] I tried to show that the pathogenesis of "congestive" splenomegaly represented a venocapillary sclerosis and that this was the result of hypertension

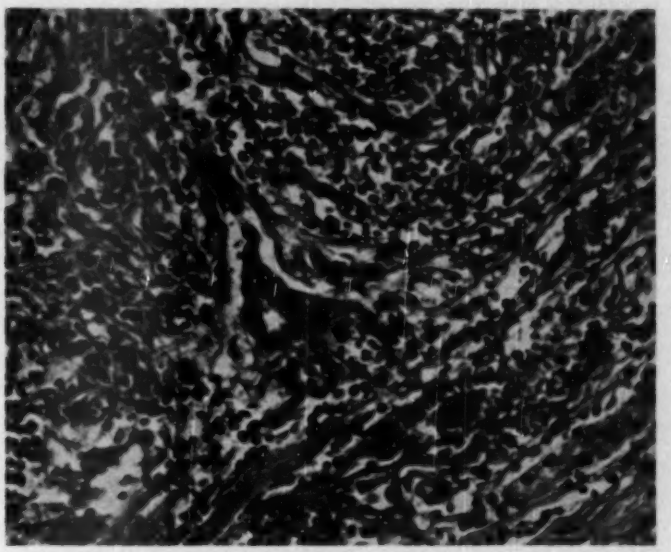

Fig. 12.—Carcinoma of lung: capillarization in lymphocytic infiltrate surrounding neoplasm.

of the portal circulation. In the evolution of this process I showed that there was an early fibroblastic proliferation in the Billroth cords which contain the labyrinthine spaces that are the analogues of the capillaries in other organs. Later, new splenic sinuses form which contribute almost entirely to the enlargement of the organ. In time, if the hypertension of the portal circuit continues, sclerosis occurs, with almost complete obliteration of the splenic pulp cords, and the circulation within the organ is converted from an open to a closed one.

48. Moschcowitz, E.: Medicine **27**:187, 1948.

It is apparent that the mechanism of the formation of new sinuses is comparable to that which I have submitted in the production of all other forms of chronic granuloma and that one may ascribe to the splenic pulp the pluripotential properties of mesenchyme.[49] Teleologically, the sinus hyperplasia may be viewed as compensatory to the destruction of the capillary bed. I have already commented on the presence of extramedullary hematopoiesis in protracted cases of "congestive" splenomegaly and the belief that it represents a phylogenetic revision.

Hodgkin's Disease.—In this condition, which is universally viewed as a form of granuloma, the cellular elements, consisting of round cells, plasma cells, eosinophilic granulocytes and giant cells, I have noted capillarization in the fibroblastic areas.

I do not presume to have considered all types of chronic inflammation. For instance I have omitted the central nervous system, the osseous system, the musculature, the skin, etc. But I do feel that I have submitted enough to prove my thesis, namely, that autochthonous angiogenesis occurs in practically all forms of granuloma and that it contributes to a greater or lesser extent to the healing process, with resulting collagenous and sclerotic transformation. In other words, one of the functions of the lymphocyte is its potentiality for forming endothelium and therefore for angiogenesis, and subsequently, when the new vessels are no longer required, they are transformed to collagen and sclerotic connective tissue.

SUMMARY

Evidence has been submitted that in a wide variety of chronic granulomas autochthonous formation of new blood vessels is the rule and that it occurs in the course of a fibroblastic differentiation of the newly formed mesenchyme. This mesenchyme is formed primarily from lymphocytes that exude from blood vessels and to a lesser extent from fixed connective tissue cells which undergo a transformation. The mechanism of the angiogenesis represents a reversion to that which occurs in the embryo. The hyperplasia of the sinuses and the extramedullary blood formation in "congestive" splenomegaly are explainable in this interpretation. One of the functions of the lymphoid cell in chronic inflammation is its potentiality for being transformed to endothelium, and hence for angiogenesis. The newly formed vessels either persist or become destroyed. In the latter case the component cells revert to collagen and eventually to sclerotic connective tissue. The whole is viewed as part of the reparative process inherent in the concept of inflammation.

49. Klemperer, P.: The Spleen, in Downey, H.: Handbook of Hematology, New York, Paul B. Hoeber, Inc., 1938, vol. 3.

EXPERIMENTAL FLASH BURNS

The Pathologic Aspects

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MODERATE temperature burns have been studied numerous times in the clinic and the laboratory. Henriques and Moritz [1] observed the pathologic and physical characteristics of these lesions. Gordon, Hall, Heggie and Horne [2] have followed histologically the progress of first, second and third degree moderate temperature burns in the clinic, and Leach, Peters and Rossiter [3] noted the cytologic variants in experimental moderate temperature burns. To our knowledge, no investigation has been made of the cutaneous changes produced by a high intensity, brief radiant heat stimulus. This is the "flash burn," the importance of which was emphasized at Pearl Harbor, in Hawaii, as well as by the large number of burn casualties at Hiroshima and Nagasaki, in Japan.[4] Its severity is proportional to the energy flux per unit area of exposed surface. A flash source capable of producing such a lesion must have the energy characteristics set forth previously.[4] This report describes the lesions produced in skin exposed to the firing of commercial photographic magnesium flash powder, an agent used experimentally by Fauley and Ivy [5] in the process of developing protective flash burn creams.

It was found by experimentation that 124 Gm. of magnesium powder fired at distances of 20 to 60 cm. from cutaneous targets produced flash

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This paper is based on work performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project.

1. (*a*) Henriques, F. C., and Moritz, A. R.: Am. J. Path. **23**:531, 1947. (*b*) Moritz, A. R., and Henriques, F. C.: ibid. **23**:695, 1947.

2. Gordon, J.; Hall, R. A.; Heggie, R. M., and Horne, E. A.: J. Path. & Bact. **58**:51, 1946.

3. Leach, E. H.; Peters, R. A., and Rossiter, R. J.: Quart. J. Exper. Physiol. **32**:67, 1944.

4. Pearse, H. E.; Payne, J. T., and Hogg, L.: Ann. Surg. **130**:774, 1949.

5. Fauley, G. B., and Ivy, A. C.: United States Nav. M. Bull. **43**:209, 1944.

burns of varying severity (fig. 1). Many factors influenced these burns, namely, the intensity of the energy flux governed by the distance, the amount of magnesium used, the cutaneous thickness, the anatomic position and the curvature of the exposed area. These factors were controlled as satisfactorily as possible. The physical characteristics of the magnesium flash powder used in this experiment are listed in table 1.

MATERIALS AND METHODS

Henriques and Moritz [18] have shown experimentally that porcine skin is anatomically and physiologically similar to human skin; therefore the pig was chosen as the experimental animal. Young pigs, well anesthetized and closely

Fig. 1.—An early stage of burning magnesium (approximately one hundredth of a second after firing). Note the shape of the flame. The transformer igniter and the exposure box containing a well anesthetized experimental animal are seen in the background.

TABLE 1.—*Physical Characteristics of Magnesium Flash Powder*

Charge used	124.0 Gm.
Temperature	3700 C.*
Flash time	0.34 second
Spectral qualities	Ultraviolet rays—high Visible rays—high Infra-red rays—unknown
Spectral quantities	Unknown

* Worthing, A. G.: Measurement of Radiant Energy, in Sources of Radiant Energy, New York, McGraw-Hill Company, 1927, chap. 2, p. 25.

clipped, were shielded and exposed through various-sized apertures to the burning magnesium flash source (figs. 2*A* and *B*). Immediately after exposure, all lesions were examined and graded arbitrarily according to the type and the degree

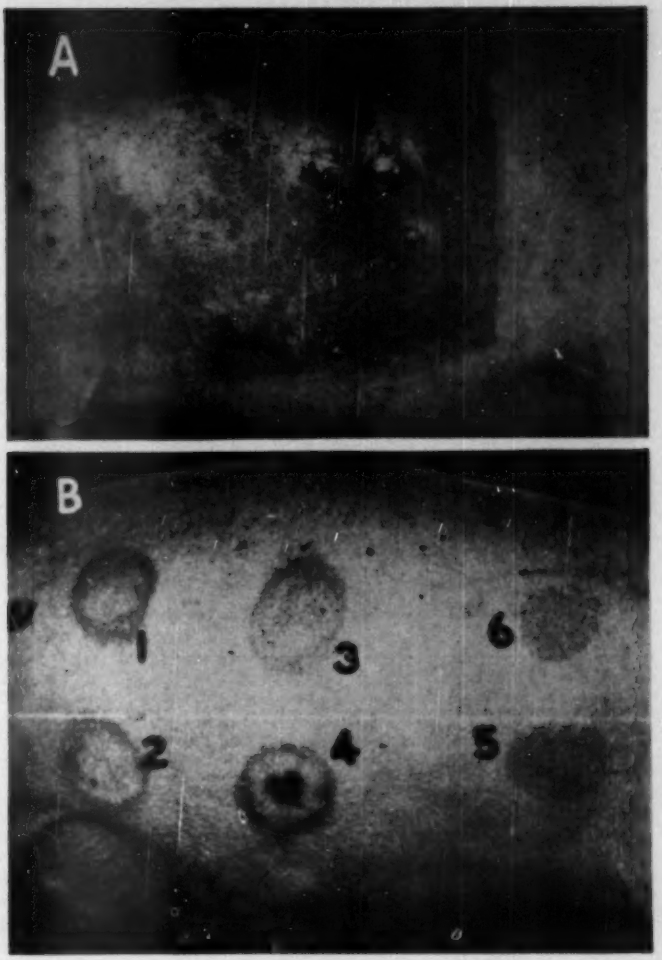

Fig. 2.—*A*, burn, 10 by 15 cm., produced through a large port in a transite shield by burning 124 Gm. of magnesium at a distance of 30.0 cm.[4]

B, variations in the severity of the burns as seen immediately after the animal was exposed through circular apertures at various distances from a constant charge of magnesium. See table 2 for details of evaluation and rating.

of damage. The observations are listed in table 2. A total score was taken for each burn and this value used to establish three grades of severity. They are listed as follows:

Type of Burn	Numerical Severity Rating
Mild	1-5
Moderate	6-8
Severe	9-17

Thirty-five millimeter kodachrome* photographs and serial biopsy specimens were taken at intervals of one-half, six, twelve and twenty-four hours, and these

TABLE 2.—*Data Recorded for Burns Produced by Exposing Porcine Skin to a Constant 124 Gm. of Burning Magnesium Flash Powder at Distances Varying from 20 to 60 Cm.**

Burn	1	2	3	4	5	6
Skin target distance, cm.	20	30	40	20	50	60
Hair damage						
Tip +)	0	0	0	0	+	+
Midshaft ++)	0	++	++	0	0	0
Root +++)	+++	0	0	+++	0	0
Epithelial damage						
Scaling +)	0	0	0	0	0	0
Vesicles ++)	0	0	0	0	0	0
Fissuring +++)	0	0	0	+++	0	0
Denudations ++++)	0	0	0	0	0	0
Vascular responses						
Erythema +)	0	0	0	0	0	+
Reddening ++)	0	0	++	0	++	0
Cherry red +++)	0	+++	0	0	0	0
Dark red ++++)	++++	0	0	++++	0	0
Blanching						
Mild +)	0	0	0	0	0	0
Moderately severe ++)	0	0	++	0	0	0
Severe +++)	+++	+++	0	+++	0	0
Edema						
Mild +)	+	0	0	+	0	0
Moderate ++)	0	0	0	0	0	0
Severe +++)	0	0	0	0	0	0
Total grade of burn	11	8	6	14	3	2

* The type and the degree of cutaneous damage and the vascular responses were recorded. The pluses indicate the degree of damage or response observed in accordance with the identifying keys in the left margin. Zeros indicate that there were no reactions of the types noted.

procedures were repeated every twenty-four hours up to two weeks. The tissues were fixed in Bouin's fluid and mounted in paraffin, and sections were stained with hematoxylin and eosin.

OBSERVATIONS

A total of 90 burns of different types were produced by varying the distances of the skin surfaces from the burning of a constant charge of magnesium powder. The lesions were studied grossly and histologically and classified into three types according to severity, namely, mild, moderate and severe.

Mild Burns.—The mild type of flash burn (fig. 2 *B*, burn 6), the initial reaction to which was erythema, underwent a progressive deepening of the erythema until the fourth or the fifth day, when the color shaded into a tan, and then a brown. The brown deepened, became spotty and gradually scaled on the tenth day.

The earliest microscopic changes seen in this lesion were in the epidermis (fig. 3). Here the juncture of the burned area and the normal skin was demarcated by changes in the epithelial cells. From the normal, basophilic epithelial cells

the transition was gradual to the heat-damaged, eosinophilic cells. The changes were similar to those described by Leach, Peters and Rossiter.[5] However, typically they consisted in flattening of the superficial cells of the stratum spinosum and nuclear pyknosis. The cells of the deeper parts of the stratum lucidum and stratum germinativum, showed nuclear shrinkage and perinuclear vacuolation. The stratum germinativum exhibited spotty areas of tonofibrillary stretching and a few areas of dermal-epidermal separation. Horizontally, superficial demarcations at the mouths of hair follicles were similar to those seen at the lateral epithelial borders. The dermal structures showed spotty areas of minimal coagulation of the collagen fibrils beneath the areas of severe epidermal damage. Vascular changes were not apparent.

Moderate Burns.—The moderate type of flash burn (fig. 2 *B*, burns 2 and 3) was characterized by a distinct gray-white, elevated, intact central island, surrounded by a narrow erythematous collar. A transitory flare appeared about this collar twenty seconds after exposure, reached a maximum intensity and spread

Fig. 3.—A section taken from the region of a mild burn six hours after exposure, showing cytologic variants of types G and H, described by Leach and associates.[6] These cells are shown in the stratum germinativum in all areas except the central one. Here, in the central depression, the stratum germinativum cells are those of the D and E type, and show perinuclear vacuolation, signifying less severe damage. × 110.

about the lesion in approximately one hour, and then subsided. These reactions were accompanied by progressive edema. One to two days after exposure the intensity of the erythema increased. The central island at this time was violaceous, soft, compressible and elevated, with an umbilicated surface.

About three days after exposure the most noticeable change was the appearance of pale golden pigmentation in the peripheral and central areas. A noticeable condensation of the lesion occurred at this time and accentuated the color. By the fifth day the island was transformed into a firm brown eschar attached to, and penetrated by, the underlying hairs. The regenerated epithelium beneath the eschar, when exposed, was thin, pale and translucent. When the eschar was removed, small hemorrhages were produced at points at which it had been attached to the underlying dermis. Between the seventh and ninth days the damaged areas were completely reepithelized.

This type of burn showed microscopically a shredded stratum corneum. The transition from burned to normal epidermis was abrupt (fig. 4 *A*) and was often accompanied by epidermal detachment at the line of juncture. The burned cells

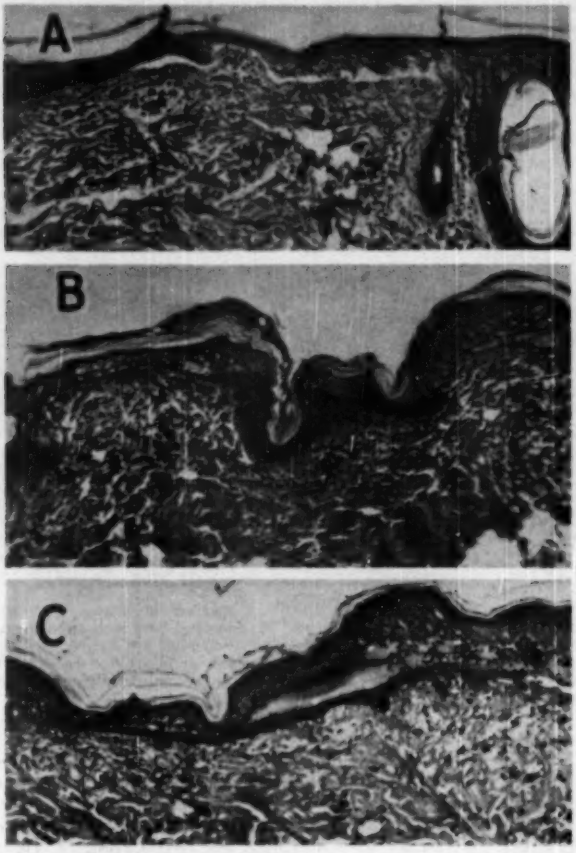

Fig. 4.—*A*, section of a biopsy specimen of the region of a circular burn taken immediately after exposure. Note the abrupt transition between the normal and the thermally injured epidermis. The injured epidermis is seen to be detached from the mildly coagulated dermis. The depth of penetration of the injury is shown by the demarcation of the hair follicle.

B, section of a biopsy specimen taken immediately after exposure, showing the abrupt horizontal demarcation of the hair follicle. × 120.

C, section taken from the region of a moderate burn two days after exposure, showing a thermally coagulated detached epidermis with underlying leukocytes and edema fluid. Laterally a new, regenerating epidermis is seen growing beneath the old, damaged epidermis. × 120.

presented various degrees of damage from simple nuclear pyknosis through cytoplasmic vacuolation to gross distortion with flattening and condensation. Various degrees of dermal-epidermal separation were present throughout the burned areas. The attached epidermis was held by stretched tonofibrils. The intertonofibrillary spaces enlarged, and edema fluid and a few red cells were seen within them. Only these areas were similar in appearance to true vesicles.

The hair follicles showed horizontal changes similar to those seen at the abrupt lateral margins of the lesions (fig. 4*B*). Striking was the abrupt demarcation between basophilic, normal germinal epithelium and eosinophilic, burned cells with pyknotic nuclei and perinuclear vacuolation or palisaded amorphous cells. The depth of this demarcation of the hair follicle layers was an index of the depth of penetration and the severity.

The dermal layer in the regions of moderate and severe burns showed injury of variable depth closely paralleling the damage of the hair follicle. The superficial fibers were swollen, amorphous, pink, and overlaid a zone of separated fibers in which the interfibrillary spaces were filled with edema fluid. These spaces were noticeably filled at six hours; by the second day the edema reached a maximum, and then regressed over the next two to four days. In these spaces, at the end of six hours a progressive polymorphonuclear leukocytic infiltration occurred concomitant with the accumulation of fluid (fig. 4*C*).

The demarcation between injured and normal surface epithelium was marked by the end of the first day; this was apparently dependent on the staining reaction of the necrotic cells. The dermal leukocytic infiltration neared a peak at this time. At twelve to twenty-four hours the smaller vessels contained clumped red cells and showed some perivascular leukocytic collaring.

By the end of twenty-four hours, some of the burned areas showed beginning epithelial regeneration at the lateral borders and at the mouths of hair follicles beneath the eschar. By the second day this was more evident (fig. 4*C*). The epithelium began as a single layer of cells, increased to two, then three cells, and finally to a full thickness of cells covering the entire area of the lesion with a new epidermis. The eschar was thus sequestered with minimal reaction (fig. 5*A*). In some areas the damaged dermal collagen was covered by the new cells. The process of reepithelization required from three to five days, although normal epidermal thickness was often not achieved in less than one week. At about the fourth day the cytoplasm of the superficial cells of the stratum spinosum showed increased basophilic granular stippling. These granule-bearing cells persisted and became increasingly superficial about the eighth or the ninth day.

There were many variations in the histologic picture. In the sections taken immediately after exposure the demarcation between normal and burned cells was seen in the skin crypts—the bottoms being preserved, the tops burned. Occasional areas healed by granulation.

Severe Burns.—The severe type of flash burn (fig. 3, burn 4) showed the same general picture as the moderate burn except that the central island of this burn showed evidence of trauma, manifested by epithelial laceration, avulsion and bleb formation. The blebs seen were filmy, parched, white membranes, partially detached, and contained a small amount of serous fluid. The course of this group differed little from that of the moderate burns.

The process of repair was similar to that observed in the moderate burn. Occasionally a severe area was seen in a large burn which showed more destructive changes on microscopic examination. In this transcutaneous lesion, splitting and partial or complete detachment of the epidermis was seen immediately after

exposure (fig. 6 *A*). Various types of coagulated epidermal cells were arranged in bizarre fashion and showed nuclear compression and distortion. In the middle and lower levels a villous nuclear pattern was rather constant. Dense acidophilic cytoplasmic coagulation and nuclear pyknosis and condensation were seen in the upper and middermal levels and occasionally in the lower level. Destruction of hair follicles was complete except for a few remaining clusters of cells in the deep layers. The glands in the subcutaneous layer survived.

After the first day the epidermal cellular pattern and characteristics were unchanged. The injured dermal layer showed a distinct pale basophilic tint and was separated by an increasing band of leukocytes. Beneath this barrier connective tissue fibers were separated by edema fluid, which contained leukocytes. A few leukocytes were seen in the glandular acini. Regeneration and proliferation of the surviving lateral epithelium occurred early, and new epithelium grew beneath the damaged tissues gradually.

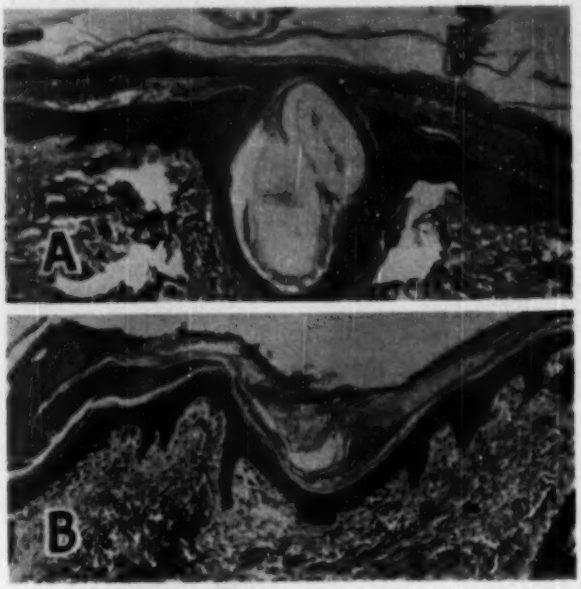

Fig. 5.—*A*, section of a biopsy specimen taken four days after exposure, showing the damaged tissue separated as a sequestrum. × 120.

B, thin layer of epidermis, 1 week old, collectively regenerated from surviving marginal, follicular and rete peg epidermis. Full thickness (7 to 10 cells) has not been achieved. Early rete peg formation is seen. × 120.

By the end of the second day an increased concentration of leukocytes was seen, and distinct demarcation of the damaged tissue was evident (fig. 6 *B*). Degeneration of the superficial leukocytes occurred. The inflammatory barrier was denser and more discrete than before. The injured dermis was represented

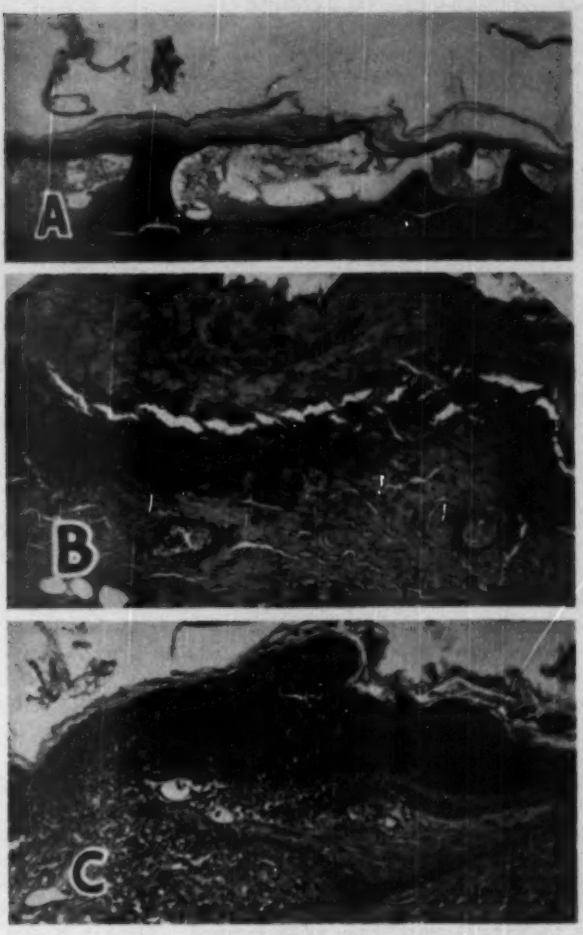

Fig. 6.—*A*, section of a biopsy specimen taken from the region of a severe burn, showing a shredded stratum corneum and epidermal coagulation with bleb formation. × 120.

B, section of a biopsy specimen taken from the region of a severe large burn two days after exposure, showing "leukocytic delineation" of the coagulated epidermis and dermis above and the surviving dermis below. × 120.

C, section taken from a severely injured part of the area of a large burn eleven days after exposure, showing granulation tissue and epithelium growing beneath the eschar. × 120.

by a distinct acidophilic coagulum with dark blue streaks. Spotty foci of deeper dermal necrosis were present. Reepithelization progressed from the lateral margins as before.

By the fifth day, hyperplasia and proliferation of the epithelium were increased. There was active propagation of the lateral epidermis, which grew beneath the coagulum. Laterally, where a thin epidermis had regenerated, rete peg formation began. Granulations beneath the injured dermal level were increased.

At the end of approximately two weeks the injured epidermis and dermis and incorporated adnexial remnants were converted into a dense basophilic atrophied eschar. The eschar was separated by the undergrowth of epithelium from the lateral margins, follicle remnants and probably from the surviving glands. In the surviving dermis, masses of granulation tissue were seen about the regenerated epithelium (fig. 6 *C*).

COMMENT

The purpose of the early phases of this work has been to find and define the differences between the low temperature burn and the high intensity, short duration flash burn. Three important differences were apparent: (1) histologically, the abrupt horizontal and lateral cutaneous demarcations; (2) grossly, the amount of edema produced, and (3) the method of healing. The abrupt lateral transition from basophilic normal epidermal cells to eosinophilic burned, necrotic cells was striking and without parallel in the moderate temperature injury. The similar abrupt demarcation seen in the skin crypts and hair follicles was equally striking and unique. One of the key points in the discussion of Leach, Peters and Rossiter [2] was the gradual transition of cell types at the border of the low temperature burn. This was not seen in the flash burn. The cellular reaction evoked by thermal injury was the same in both the high and the low intensity burns, and the cell types were as Leach and associates described them. However, their arrangement and relationship at the borders and the depths of the lesions were quite different, and the abrupt sequential arrangement laterally and in depth was specific.

The healing of the flash burn was by sequestration of the necrotic epithelium and dermis rather than by organization such as that noted with moderate temperature burns. Both the phenomenon of demarcation and the healing beneath a sequestrum are related to the conditions under which heat is transferred through the tissues. Apparently the brevity of the intense thermal assault does not permit the tissues to come to thermal equilibrium while exposed. Thus it is that the area which is exposed is injured beyond repair. Deeper tissues are virtually unaffected.

The erythema and the edema are outstanding. In the low intensity, long duration burn, the cutaneous blood vessels are thermally fixed, making the skin an ivory-hued, leathery structure, incapable of vascular

reaction. The flash burn, however, apparently produced vascular effects which left the vessels capable of reactivity immediately beneath the lesion.

SUMMARY

The importance of the flash burn was emphasized at Pearl Harbor and by the large number of burned casualties at Hiroshima and Nagasaki.[4]

Experimental flash burns were produced on swine by exposing the animals to the heat of burning magnesium flash powder.

The burns were characterized on histologic section by abrupt demarcations laterally and horizontally.

The lesions healed by sequestration or organization of the burned tissues.

The cutaneous blood vessels beneath the burns retained reactivity, so that erythema and edema were prominent.

The physical and the physiologic aspects of these lesions are under investigation.

TOXICITY STUDIES ON VITAMIN B_{12} IN ALBINO MICE

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FOLLOWING the first published reports [1] on vitamin B_{12}, many papers appeared announcing the brilliant results obtained with this drug in cases of pernicious anemia and other macrocytic anemias.[2]

The effects of vitamin B_{12} on chicks,[3] embryonated eggs [4] and rats [5] have also been studied. However, neither in man nor in animals has toxicity due to vitamin B_{12} been observed, if one may judge by the literature, although studies in this field are currently in progress at the Merck Institute.[6]

The reason for this is that very small doses of vitamin B_{12} have been used. The object of this paper is to report the effects produced by large doses of vitamin B_{12} [7] on albino mice.

PROCEDURE

Forty albino mice (median weight, 11 Gm.) were divided into four groups of 10 each and were given intraperitoneal injections of vitamin B_{12} in doses of 7.5 micrograms (group 1), 15 micrograms (group 2) and 30 micrograms (group 3). The fourth group received 30 micrograms of the drug subcutaneously.

RESULTS

The results of the administration of vitamin B_{12} are shown in the table.

From Fairview Park Hospital.

1. Rickes, E. L.; Brink, N. G.; Koniuszy, F. R.; Wood, T. R., and Folkers, K.: Science **107**:396, 1948. Shorb, M. S.: ibid. **107**:397, 1948. West, R.: ibid. **107**:398, 1948.

2. Hall, B. E., and Campbell, D. C.: Proc. Staff Meet., Mayo Clin. **23**:584, 1948; ibid. **23**:591, 1948. Spies, T. D.; Garcia Lopez, G.; Milanes, F.; Lopez Toca, R., and Culver, B.: South. M. J. **41**:523, 1948. Spies, T. D.; Stone, R. E.; Garcia Lopez, G.; Milanes, F.; Lopez Toca, R., and Aramburu, T.: Lancet **2**:519, 1948.

3. Ott, W. H.; Rickes, E. L., and Wood, T. R.: J. Biol. Chem. **174**:1047, 1948. Schaefer, A. E.; Salmon, W. D., and Strength, D. R.: Proc. Soc. Exper. Biol. & Med. **71**:202, 1949.

4. Wagley, P. F., and Morgan, H. R.: Bull. Johns Hopkins Hosp. **83**:275, 1948.

5. Hartman, A. M.; Dryden, L. P., and Cary, C. A.: Arch. Biochem. **23**:165, 1949.

6. Mushett, C. W.: Personal communication to the author.

7. The preparation of vitamin B_{12} marketed under the name of rubramin® was used and was supplied for this investigation by E. R. Squibb & Sons, 745 Fifth Avenue, New York 22.

From these findings we can say that in the albino mouse an intraperitoneal dose of about 0.75 mg. per kilogram is devoid of toxicity, while an intraperitoneal dose of 1.5 mg. per kilogram is decidedly toxic, and a dose of 3 mg. per kilogram, administered either intraperitoneally or subcutaneously, is lethal for 100 per cent of the animals treated. Necroscopy showed, on gross inspection, only congestion and edema of the lungs and slight congestion of the visceral organs. Histologic study of liver, kidney, adrenal gland, intestine, thymus, testicle and spleen did not show any pathologic change. The lungs presented intense hyperemia.

The possibility that there was allergy to vitamin B_{12} in the guinea pigs was also studied. The technic employed was the same as that used by Gruhzit and Fisken in their study concerning diphenhydramine hydrochloride (benardyl hydrochloride® N.N.R.).[8] Twelve guinea pigs (median weight, 400 Gm.) were employed. Each was given subcutaneously 5 micrograms of vitamin B_{12} daily for twenty-one days. After an interval of thirty-seven days of rest each animal received 15 micrograms

Data Concerning Experimental Administration of Vitamin B_{12}

Group	Animals Treated	Route of Injection	Amount of Vitamin B_{12}, Micrograms	Signs of Toxicity	Mortality Rate
1	10	Intraperitoneal	7.5	None	None
2	10	Intraperitoneal	15	Excitatory state with convulsions	2/10
3	10	Intraperitoneal	30	Excitatory state with convulsions ending in cardiac and respiratory failure	10/10
4	10	Subcutaneous	30	Excitatory state with convulsions ending in cardiac and respiratory failure	10/10

of vitamin B_{12} intraperitoneally. None of them showed reactions immediately or subsequently. These experiments, although limited, show that in the guinea pigs so treated no sensitivity developed. In addition, it is important to point out that in a patient allergic to liver extracts the injection of vitamin B_{12} did not cause an allergic reaction[9] and that vitamin B_{12} has been shown to possess antianaphylactic and antihistaminic activity.[10]

SUMMARY

Vitamin B_{12} is shown to be toxic to albino mice when administered intraperitoneally and subcutaneously in very large doses. Mice that died after injection of a preparation of this vitamin showed no pathologic changes other than congestion of the lung. Guinea pigs did not exhibit sensitivity to vitamin B_{12}.

8. Gruhzit, O. M., and Fisken, R. A.: J. Pharmacol. & Exper. Therap. **89**:3, 1947.

9. Berk, L.; Denny-Brown, D.; Finland, M., and Castle, W. B.: New England J. Med. **239**:328, 1948.

10. Traina, V.: Unpublished data.

OVARIAN CANCER IN MOTHER AND FIVE DAUGHTERS

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OVARIAN cysts and tumors occurring in more than one member of a single family were reported as early as 1877 by Olshausen.[1] This and several other early reports have not been located and are cited from the brief mention accorded them by later authors.[1] Löhlein [2] reported a family in which 3 sisters had cysts of the ovaries, histologically unidentified; Martin,[3] two pairs of sisters with ovarian tumors, unidentified; Twinem,[4] ipsilateral ovarian cysts in identical twins; Mandelstamm,[5] three families with multiple dermoid cysts in sisters; Barnes,[6] chocolate cysts in 2 sisters. Reports of familial occurrence of serous and pseudomucinous cysts published prior to 1946 were reviewed by Selye.[7]

Published accounts of familial ovarian growths that were proved to be cancers are exceedingly scarce.

Waaler [8] found that the sisters of patients with cancer of the breast, uterus or ovaries suffered from more cancer than their brothers, and tumors of the type in the patient were more frequent in the sisters than in the sisters of women with cancers of other organs. In the abstract available, numbers are given for cancers of the breast and uterus but not for those of the ovaries.

From Julius Wasserman Laboratories of Montgomery County, Amsterdam City Hospital, and St. Mary's Hospital, Amsterdam, N. Y.

1. Olshausen, R. M.: Die Krankheiten der Ovarien, Stuttgart, F. Enke, 1877 and 1886, cited by Löhlein [2]; Köberlé, cited by Löhlein [2]; Péan, cited by Martin [3]; Pfannenstiehl, cited by Mandelstamm [5]; J. Y. Simpson, cited by Martin [3]; Sippel, cited by Mandelstamm [5]; Winckel, cited by Martin.[3]

2. Löhlein, H.: Monatsschr. f. Geburtsh. u. Gynäk. **3**:91, 1896.

3. Martin, H.: Handbuch der Krankheiten der weiblichen Adnexorgane, Leipzig, E. Besold, 1899, vol. 2, p. 372.

4. Twinem, F. P.: New York State J. Med. **27**:1192, 1927.

5. Mandelstamm, A.: Zentralbl. f. Gynäk. **53**:2356, 1929.

6. Barnes, J.: Proc. Roy. Soc. Med. **38**:324, 1945.

7. Selye, H.: (*a*) Ovarian Tumors, in Encyclopedia of Endocrinology, Montreal, Richardson, Bond & Wright, 1946, sect. 4, vol. 7, p. 163 L; (*b*) ibid., p. 162 R.

8. Waaler, G. H. M.: Ueber die Erblichkeit des Krebses, Skrifter Norske Videnskabsselskabet, Oslo Akademie I. Matematisk-natur-Videnskabelig Klasse Skrifter, 1931, no. 2, p. 78; abstracted, Cancer. Rev. **7**:464, 1932.

Kimbrough [9] reported bilateral papillary adenocarcinoma of the ovaries in apparently uniovular twins. In both the first symptoms appeared at the age of 39, and the carcinomas were solid and bilateral.

Abruzzese [10] found that relatives of patients with female genital tumors had homologous tumors twice as frequently as the general population, while the incidence of tumors of any of their other organs was the same as that in the general population. This familial incidence was most obvious in regard to ovarian cystocarcinoma, and was present in all cases of bilateral cystocarcinoma. No precise numbers or genealogies are given, and there is no histologic illustration, description or definition of cystocarcinoma. Familial incidence was not found in the presence of noncancerous cysts of the ovary.

Lynch [11] studied 110 cases of ovarian cancer of different types. A positive history of cancer in the family was given by 42 patients. Of the relatives who were stated to have died of cancers of known sites, those with ovarian cancers were distributed as follows: mothers, 2; sisters, 1. One mother, 1 aunt, 1 sister each had "abdominal" cancer. Lynch did not state the histologic types of the ovarian cancers in the 42 patients who reported that relatives had suffered from cancerous growths. But it is probable that most of them were papillary carcinoma or adenocarcinoma, as these two together made up 95 per cent of the total of 110 cancers. The author stated "No other group of pelvic cancers studied in my clinic have given such a high percentage of positive family history, although, of course, 110 cases are far too few to afford any definite data."

Montgomery [12] reviewed the 119 cases of ovarian cancer in which treatment was recorded between 1921 and 1946. Of 107 cases in which there was no doubt of the diagnosis and histologic sections were available, 84 were cases of primary adenocarcinoma of the ovary. Family histories were recorded in 75 cases, in 10 of which "a family history of cancer" was given. The author stated "such figures may be unreliable since the accuracy of the patient's knowledge of her family medical history is often open to question."

All the authors whose reports have been reviewed thus far have found some evidence of familial incidence of ovarian adenocarcinoma. A dissenting opinion was expressed by Selye [7]: "In general known simultaneous tumor formation is rarely seen in pairs of identical twins and as judged by the evidence in the section on 'Racial Incidence' there is little to support the view of an important hereditary factor in the development of common ovarian carcinomas."

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9. Kimbrough, R. A.: Am. J. Obst. & Gynec. **18**:148, 1929.
10. Abruzzese, G.: Riv. ital. di Ginec. (supp.) **17**:1, 1935.
11. Lynch, F. W.: Am. J. Obst. & Gynec. **32**:753, 1936.
12. Montgomery, J. B.: Am. J. Obst. & Gynec. **55**:201, 1948.

In only one report,[9] that of Kimbrough's pair of uniovular twins, is it stated that the multiple cases within one family had been observed directly by the author, with histologic material available. The other reports are based on family histories obtained from patients or on statistical studies.

In answer to requests for information, Dr. M. Demerec, of the department of genetics of the Carnegie Institution, Washington, D. C., stated that they had done no work on the subject of hereditary factors of tumors of the ovary (May 1944) and Dr. Emil Novak, of the Ovarian Tumor Registry, stated that they had no case which illustrates any familial tendency (April 1944).

In 1936, Furth and Butterworth[13] reported that ovarian tumors had been induced in mice by radiation. Furth and Boon[14] found no evidence of genetic factors or of variable susceptibility of strain in these mice. Li and Gardner[15] observed no strain limitations in tumors of intrasplenic ovarian grafts of mice produced by the method of Biskind and Biskind.[16] None of the tumors induced in mice was analogous to human serous papillary adenocarcinoma of the ovary.

REPORT OF CASES

The present report deals with a family in which papillary adenocarcinoma of the ovary, of a highly malignant grade, has been observed in 5 sisters and their mother. Data are available on five generations. Concerning the first generation, information is limited to the maternal grandparents, of whom the grandmother is said to have died of "old age" at more than 100, and the grandfather of cancer of the lip.

One daughter of this couple died when 73 years of age, of cancer of the ovary (case 1), and was the mother of 5 sisters who had ovarian cancers (cases 2 to 6). She had 2 half-sisters, both dead, about whom little or nothing is known. The husband of this woman died at the age of 73 of unidentified heart disease. One of his brothers, who died after his eightieth year, had "many children," none of whom were known to have ovarian or other tumors. No data are available about a second brother and a sister.

The third generation, consisting of the offspring of the patient of case 1, comprised 12 persons. One, whose sex is unknown, died in infancy. Of the remaining 11 (4 males and 7 females), 5 have died of ovarian carcinoma (cases 2 to 6).

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13. Furth, J., and Butterworth, J. S.: Am. J. Cancer **28**:66, 1936.

14. Furth, J., and Boon, M. C.: Cancer Research **7**:241, 1947.

15. Li, M. H., and Gardner, W. U.: Cancer Research **9**:35, 1949.

16. Biskind, M. S., and Biskind, G. R.: Proc. Soc. Exper. Biol. & Med. **55**:176, 1944.

One of the 2 living sisters, aged 60, has had vaginal bleeding but has refused pelvic examination. The other, aged 49, when last heard from, in 1946, had no symptoms attributable to ovarian carcinoma.

The fourth generation was originally made up of 13 persons, of whom 5 were females. They were the offspring of 7 members of the third generation, including 4 of those who died of cancer of the ovary. The oldest of the 5 females died when 31 years of age, of heart disease; 1 died at birth, and 1 of the remaining 3 has 2 children.

The salient clinical and pathologic features of the 6 cases are listed in the table. In cases 2 and 5 sections of ovary were available. In cases 1, 3 and 6 no ovarian tissue was excised, the sections being taken from omentum. All showed the same histologic type of cancer. It is highly

Data on 6 Cases of Ovarian Cancer

Case, Relationship, Age and Year of Onset	Duration from 1st Symptoms to		First Symptoms	Extent of Tumor at Laparotomy	Ascites	Roentgen Therapy		
	1st Examination	Death				Treatments	Over-All Duration	Roentgens
1, mother 75, 1942	Few days	11 mo.	Incarcerated umbilical hernia	Pelvic peritoneum, sigmoid serosa	Present	0		
2, 1st daughter 51, 1936	4 mo.	9 mo.	Abdominal enlargement	Entire peritoneum, omentum	Present	0		
3, 2d daughter 54, 1944	3 mo.	11 mo.	Mass in lower part of abdomen, distention, dyspnea, loss of weight	Entire peritoneum, omentum	Present	32	25 weeks	10,220
4, 5th daughter 36, 1926	2 mo.	12 mo.	Abdominal pain, pelvic pressure, backache, loss of weight	Adnexa uteri, both sides; omentum, intestinal serosa	Present	0		
5, 7th daughter 38, 1943	2 mo.	3 yr.	Abdominal distention, pelvic fullness, urinary frequency, amenorrhea	Right ovary, ileal and sigmoid serosa	Present	47	30 weeks	6,820
6, 9th daughter 58, 1947	Few days	1 mo.	Abdominal pain, bloated feeling, vomiting	Entire peritoneum, omentum, mesentery, adhesions over uterus and right ovary	Present	0		

improbable that this type could have originated in an organ other than the ovary. Calcospheres were seen in the stroma of omental implants in cases 1 and 3, an observation which further confirms the ovarian origin of the tumors, as these structures are almost never seen in other cancers occurring in the abdomen. In case 4, unfortunately, neither section nor tissue was available to me, as both were discarded by a predecessor many years ago. The pathologic report available, however, diagnoses the condition presented in a biopsy specimen of omentum as adenocarcinoma. Furthermore, in this case, as in all the others, the multiple peritoneal seeding, together with ascites, and the absence at operation of evidence of an extrapelvic primary tumor are all strong presumptive evidence of the ovarian origin of the neoplasm. It was in this patient that the ovarian carcinoma of the family first developed, before it appeared in her mother and 2 older sisters. Hence there was no way of suspecting a familial incidence. Had it not been for the fortunate acci-

dents that in all 6 cases the tissue specimens were examined in the same laboratory, that the records of all the patients and the sections of all the cancers but one were available to the same observer, and that his

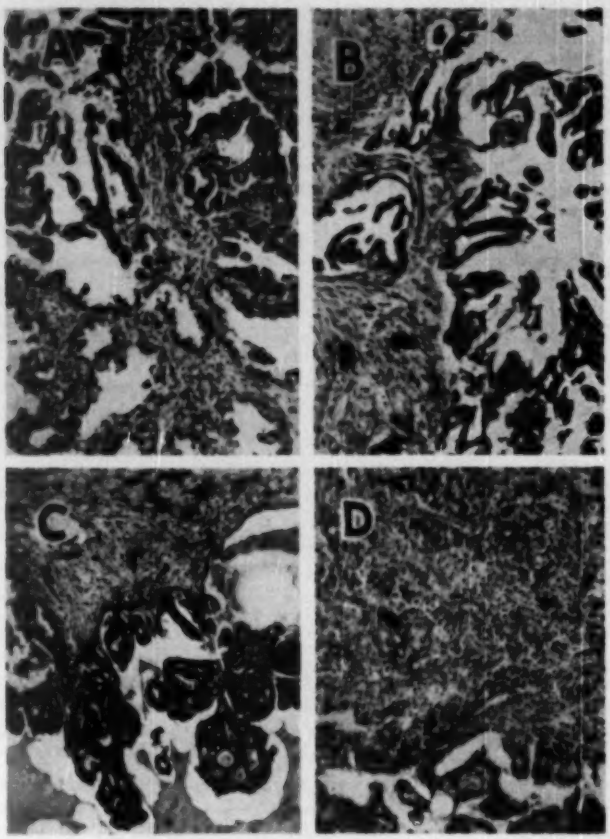

Fig. 1.—*A*, microcystic and papillary growth patterns (case 5). × 50.
B, section showing papillae extending into a cavity within the tumor, while tube-forming cells invade the stroma (case 1). Two calcospheres are visible. × 50.
C, least cancerous-appearing area showing an intratumoral cyst with microcystic papillary ingrowths (case 3). × 50.
D, solid and papillary growth (case 2). × 100.

attention was called to the existence of such a family by the surgeon, it would have been impossible to bring the cases together for study.

In the 5 instances in which microscopic sections were available the carcinoma showed extensive growth and invasion. It was arranged in a characteristic papillary pattern. The fibrovascular stroma was covered by epithelial cells, usually arranged in a single layer. In places these cells were cubical or prismatic in shape. Elsewhere they assumed greatly varied and sometimes fantastic shapes. Cilia were not seen. The cytoplasm stained a deep pink and sometimes contained irregular granules. Vacuoles were not seen. Nuclei were exceedingly varied in size and shape; some were large and monstrous. Nucleoli were prominent. Mitotic figures were extremely numerous and often of atypical character. There were solid areas of undifferentiated cells without visible

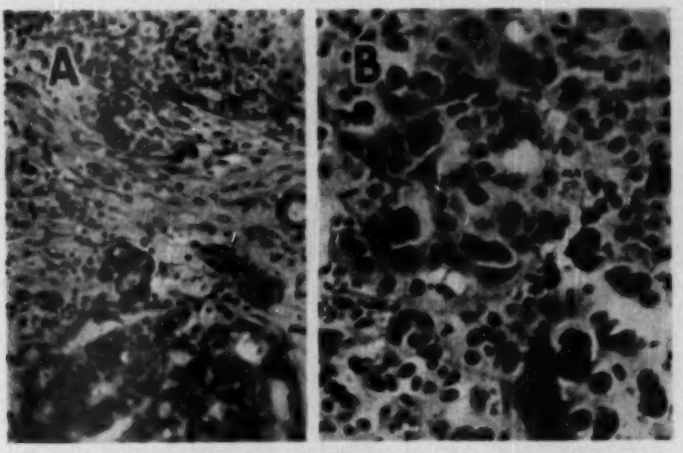

Fig. 2 (case 3).—*A*, solid and tubular growth. The stroma contains concentric calcospheres and is invaded by small epithelial tubes. × 100. *B*, detail of solid portion showing variations in nuclei. × 400.

stroma. These sheets of cells ended in papillae that projected into cavities or clefts formed within the tumor. The advancing edge of the tumor usually consisted of typical papillae.

HYPOTHESES OF TRANSMISSION

The literature concerning human cases of ovarian cancer does not provide enough material on which to formulate any working hypothesis. Thus Kimbrough's [9] 2 cases in which the patients were identical twins, with no information as to other relatives, can only suggest a germinal determination of the carcinoma. But to be more than suggestive, data concerning one egg twins must be compared with the findings in two

egg twins and in the general population. Abruzzese's report [16] indicates a frequent familial incidence but does not supply sufficient data to allow conclusions as to the mode of transmission.

The observations reported in the present paper of papillary adenocarcinoma of the ovary occurring in a mother and 5 of her 7 daughters suggest several hypotheses.

1. Chance coincidence. The computation of the chances of any given number of members of a family having a specified condition is subject to innumerable pitfalls, the main ones being the increased likelihood of cases being reported in families where similar conditions have occurred, and the unreliability of figures concerning the incidence of the condition in the general population. The interpretation of cancer statistics has been discussed recently by Dorn.[17]

The figures used here are those of the average annual incidence rates of ovarian carcinoma per hundred thousand of population in New York state exclusive of New York city for the years 1942 to 1944. They were supplied by Dr. James J. Quinlivan, of the New York State Department of Health. The average for all age groups is 12.21. All the cases studied here fall into the five year age groups 35 to 39, 50 to 54 and 70 to 74, inclusive. The age group 65 to 69 has the highest incidence, with 38.77 per hundred thousand. It is almost certain that statistical incidence rates for cancer derived from data other than autopsies are too low. This is particularly true of ovarian carcinoma. Judging from autopsies, one might conclude that it is frequently diagnosed as cirrhosis of the liver or cardiac decompensation. If, then, one takes the age group with the highest incidence, 65 to 69, as typical and uses 40 instead of 38.77, the error of this approximation is probably on the correct side, and increases the severity of the figures as a test of any hypothesis other than chance. This would mean that in any one year there would be 40 cases of ovarian carcinoma per hundred thousand population, or approximately 40 cases per fifty thousand women, an incidence of 1 in 1,250. The cases reported here all occurred within a period of twenty-one years (1926 to 1947). The chances of one woman having ovarian carcinoma over a period of twenty-one years would then be 1 in $1,250 \div 21 = 59.5$. The chances of 6 unselected women would be 1 in $59.5^6 = 1$ in 4.4×10^{10}.

In spite of the uncertainties of these computations, it is clear that the probability that chance coincidence explains the concurring of 6 cases in the present family is extremely remote.

2. Transmissibility. In the adequately studied transmissible neoplasms of short-lived animals, multiple genes, incomplete dominance, variable penetrance and extragerminal factors are the rule. Such com-

17. Dorn, H. F.: Pub. Health Rep. **59**:33, 65 and 97, 1944.

plexity could easily make impossible any valid conclusion as to the mode of transmission based on a small number of generations, with adequate data lacking on many members of the strain, as in the present family, and in the majority of the records concerning the incidence of human familial cancer. "At the present time, from ancestry data it is impossible to give the probabilities that a complex-multiple-factor trait will appear in an individual of a genetically heterogeneous population, such as the average human population" (Andervont [19]). The absence of autopsies, either of the patients with cancerous growths or of those clinically unaffected, may withhold crucial information. In the present family, no one that died has been submitted to an autopsy. For these reasons, the following possible modes of transmission must be given only as working hypotheses.

(*a*) A simple dominant gene could account for the trait appearing in the mother (case 1) and in several of her children. As the maternal grandmother is said to have lived more than 100 years, in all probability she did not have ovarian carcinoma. Hence the trait was a mutant in the mother, or it showed variable dominance, or it was acquired from the maternal grandfather. If the trait can be transmitted through males, it could, although dominant, appear to behave recessively so far as it could skip one or more generations, unless some way were discovered to detect male carriers.

The daughters of a woman with dominant ovarian carcinoma would usually have one chance in two of being attacked by the disease. There would be no way of predicting the chances that carcinoma of the ovary will occur in the daughters of a man belonging to an ovarian cancer family unless he already had one or more affected daughters; in this case the other daughters would usually have one chance in two. The hypothesis of dominance would be confirmed if in a sufficient number of families half the sisters reaching cancer age had ovarian carcinoma and half of the mothers of affected daughters had the disease, while in the cases in which the mothers did not have ovarian carcinoma the trait was present in half of the paternal grandmothers of the patients.

(*b*) A simple recessive gene could account for the facts in the present family if the mother were homozygous, and the father heterozygous—a combination likely to be rare. The children would have an even chance of being homozygous and the daughters of being cancerous. All would be capable of transmitting the trait, but the phenotype would appear in their offspring only in the rare matings between two carriers of the recessive gene. The usual patient with ovarian carcinoma would then not have the history that the neoplasm had occurred also in her mother, and rarely that it had occurred in her other female ascendants. An

18. Footnote deleted.

19. Andervont, H. B.: Pub. Health Rep. **54**:1512, 1939.

average of one quarter of her sisters would be affected. It is clear that, except in places where large families are customary, the hereditary nature of the condition would be obscured unless the gene frequency were exceedingly high, as in diabetes.

(*c*) A factor in the ovular cytoplasm might conceivably explain the present data, though the mother would be more likely to be unaffected, as the phenotype would usually be determined by the genotypes of the mother's grandparents.

(*d*) Multiple genes: Regarding animal cancers, this hypothesis was first suggested by Tyzzer,[20] in 1907, and confirmed by Little,[21] Bittner,[22] Heston, Deringer and Andervont,[23] MacDowell and Richter [24] and Cole and Furth.[25] Bauer [26] suggested that in man two genetic factors are involved: one for cancer susceptibility and one for organ localization. The present data could be accounted for by multiple genes, but are inadequate to establish this hypothesis.

(*e*) Extragerminal factors: Since Bittner [22] discovered that the carcinoma of the mouse breast is transmitted through a milk-borne virus-like factor, this possibility must be taken into consideration in all cases in which apparently hereditary traits have not as yet been proved to be transmitted exclusively by genes. That the cells of the mammary glands are not the only ones capable of being stimulated to disordered growth by a milk-borne influence was shown in Law's report [27] of a milk factor differing from the virus of the breast cancer and producing leukemia in mice. No cancer virus has been observed in man. Gross [28] has emphasized the difficulty of demonstrating this type of transmission from one generation to the next, which he termed a vertical epidemic. But, if the virus were transmitted through other mediums than milk, if contagion required many years of proximity and if the incubation were sufficiently long, familial incidence might occur although the virus was derived from an unrelated person or from any relative. It might then spread in any direction as well as vertically. Thus the father might have infected his wife and daughters, or the daughter who first had ovarian

20. Tyzzer, E. E.: J. M. Research **17**:199, 1907.

21. Little, C. C.: Science **78**:465, 1933.

22. Bittner, J. J.: Cancer Research **4**:159, 1944.

23. Heston, W. E.; Deringer, M. K., and Andervont, H. B.: J. Nat. Cancer Inst. **5**:287, 1945.

24. MacDowell, E. C., and Richter, M. N.: Zentralbl. f. Biol. **52**:266, 1932; Arch. Path. **20**:709, 1935.

25. Cole, R. K., and Furth, J.: Cancer Research **1**:957, 1941.

26. Bauer, J.: Constitution and Disease, ed. 2, New York, Grune & Stratton, Inc., 1945.

27. Law, L. W.: Cancer Research **4**:257, 1944.

28. Gross, L.: Cancer Research **4**:293, 1944; Surg., Gynec. & Obst. **88**:295, 1949.

cancer (case 4) might have been the first to acquire the virus, and her mother and sisters might have received it from her. This would explain the order in which the carcinoma affected the family, and the fact that the mother had it at a more advanced age than her daughters. The virus hypothesis would also be in keeping with the fact that frequently only one known case occurs in a family, while occasional families have multiple cases—although this could also be accounted for genically.

Any number of generations might be skipped between one case and the next if, as with the mouse milk virus, infected individuals may transmit the virus without contracting the tumor. In the present family, it has been impossible to find out whether the patients have been breast or bottle fed.

APPLICATIONS

For the present, it is impossible to foretell the chances that women of an affected family will have cancer of the ovary. One may make only the general statement that the cancer is more likely to develop in a close relative of a patient than in others. Without more information, it would be unjustified to attempt eugenic limitation of breeding. But, in the family presently under discussion is seen one of the few instances in which it should be possible to introduce effective measures of preventing cancer in the individual, or to diagnose it during a curable phase, on the basis of family history, and this is, as M. T. Macklin [29] has emphasized, the main benefit one may expect from the genetic study of cancer. Two practical measures are suggested: (1) frequent gynecologic examination starting well before the age when carcinoma is likely to appear—in this family, 35; (2) preventive oophorectomy. Since there is evidence that papillary carcinoma of the ovary may arise from misplaced rests of Müller's duct (Barzilai [30]; Ewing [31]) and since such rests occur not only in the ovary but also in the tube and the mesosalpinx, these two organs should be removed as well as the ovaries.

SUGGESTIONS FOR FUTURE WORK

Each case of ovarian carcinoma should be the occasion of a complete family study and a lifetime follow-up of all relatives. All data should be filed in one central agency, such as the Ovarian Tumor Registry or the Eugenics Record Office.

Strenuous efforts should be made to obtain permission to make an autopsy in every case of ovarian carcinoma, not only on the patient but

29. Macklin, M. T.: West. J. Surg. **50**:439, 1942.

30. Barzilai, G.: Atlas of Ovarian Tumors, New York, Grune & Stratton, Inc., 1943.

31. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940.

on every relative of the patient. This includes the males, for it is essential to discover whether there is a recognizable phenotype of male carriers.

The fact that tumor implants occasionally disappear after removal of the primary tumor, usually associated with a bilateral oophorectomy, suggests a hormonal influence which requires investigation.

The tumors and various products of the patient, such as milk, saliva, blood and cyst fluids, should be tested for carcinogenic action.

Of great practical value would be the search for a sign whereby the case of carcinoma could be detected before the tumor developed, for thus preventive oophorectomy could be limited to those known to be in danger.

If the primary tumor were found to be consistently unilateral and the precancerous ovary had a recognizable feature, such as a cyst, the operation could be limited to that ovary.

The extent to which adenocarcinoma of the ovary is preceded by noncancerous cysts remains to be established. Thus Abruzzese [16] concluded that the "heredity" of "cystocarcinoma" is direct and not via a simple "cystoma" which undergoes cancerization. In 1 case bilateral cystocarcinoma was found two years after a laparotomy in the course of which no ovarian cysts were observed. On the other hand, Selye [7b] stated, "It is now well established that cystic carcinomas do not develop primarily but only as a result of malignant transformation of originally benign cystomas."

SUMMARY

In one family, a mother and 5 of her 7 daughters died of a highly malignant papillary adenocarcinoma of the ovary that spread rapidly to the entire peritoneum.

Information is available on five generations of this family. These data and others found in the literature suggest a significantly high familial incidence but are not sufficient to permit a conclusion as to the mode of transmission. If gene transmission could be proved, a dominant gene, transmitted through men and women, could account for the data, but other modes of transmission cannot be excluded on the basis of present information.

Attention was first called to the family by Dr. Lew H. Finch, of Amsterdam, N. Y. Additional clinical data were supplied by Dr. J. A. Dickson, Mrs. Josephine McCaffrey, R. N., Dr. James J. Quinlivan and Dr. Robert C. Simpson; sections and the history of case 6, by Dr. Bayard S. Herr; the dosage of radiotherapy, by Dr. Henry K. Blake and the late Dr. David Wilson. Aid in collecting literature was given by Mr. Heath Babcock of the New York State Medical Library.

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DIRECT EVIDENCE FOR THE ANTIGENICITY OF THE GLOMERULI IN THE PRODUCTION OF NEPHROTOXIC SERUMS

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THAT glomerulonephritis similar morphologically to that which occurs in man can be produced experimentally in animals by the use of nephrotoxic serums has long been known. The exact component of the kidney which serves as antigen in the production of such serums was unknown. Recently, however, Pressman, Hill and Foote [1] demonstrated by means of radioautographs that anti-rat-kidney serum localizes specifically within the glomeruli of rat kidneys. In order to demonstrate this, the globulin fraction of the antiserum was combined with radioactive iodine (I^{131}). While the present work was nearing completion, a report by Solomon, Gardella, Fanger, Dethier and Ferrebee [2] lent additional indirect evidence that the antigenic component of the kidney lies within the glomerulus. They found that the nephrotoxic activity of anti-rat-kidney serum was nullified when adsorbed with isolated, highly purified glomeruli. On the other hand, the activity of the antiserum was retained when it was adsorbed either with other portions of rat kidney or with several different types of rat tissues.

The present report presents a simple method for isolating large numbers of glomeruli free of other renal components. It also demonstrates that by using glomeruli only, as antigen, a potent nephrotoxic serum can be obtained, whereas the remaining components of the renal cortex are ineffective in producing such serum.

PROCEDURE

The kidneys of normal dogs were removed aseptically and perfused with sterile isotonic solution of sodium chloride. They were stored at —25 C. All procedures were performed in a sterile room equipped with ultraviolet lamps. All equipment

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1. Pressman, D.; Hill, R. F., and Foote, F. W.: Science **109**:65, 1949.

2. Solomon, D. H.; Gardella, S. W.; Fanger, H.; Dethier, F. M., and Ferrebee, J. W.: J. Exper. Med. **90**:267, 1949.

was sterile and was handled with sterile gloves. When used, the kidneys were reperfused, stripped of their capsules, and the medulla of each was separated from the cortex and discarded.

Preparation of Whole Cortex.—The cortex was cut into fragments and ground for two to three minutes in an ice-jacketed Waring blendor. The pulp was washed three to five times with sterile isotonic solution of sodium chloride by centrifugation. The sediment contained fragments of all cortical components, including largely intact glomeruli. This was suspended in enough saline solution to make a thin paste. Each cubic centimeter of paste contained on the average 45,050 glomeruli.

Preparation of Glomeruli.—With the handle of a scalpel, fragments of the prepared cortex were forced through a stainless steel sieve of 100 mesh into a mortar kept on ice. This paste was then collected into a plastic tube and diluted with 82 per cent sterile sucrose solution (specific gravity, 1.34). It was then spun

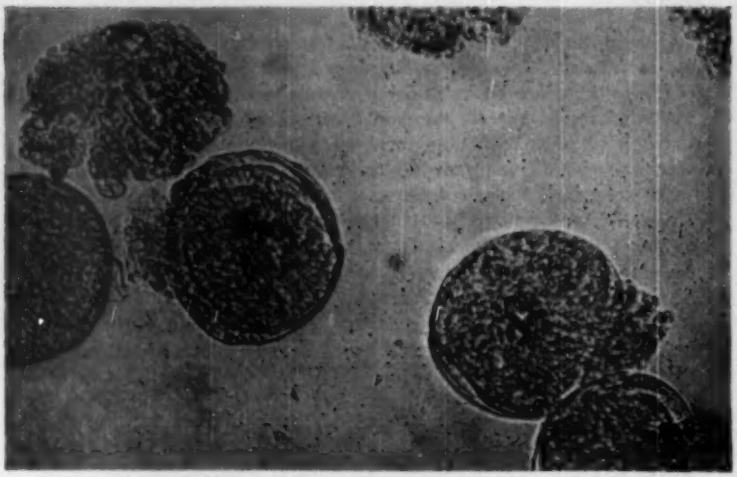

Fig. 1.—Isolated glomeruli with and without parietal capsules. × 200.

in a refrigerated high speed centrifuge at 18,000 revolutions per minute (r.p.m.) for three to five minutes. The intact isolated glomeruli were thus brought to the top and could readily be seen with the naked eye, forming a layer on the surface and an angulated cone sloping somewhat deeply into the solution toward one side of the wall of the tube. Mixed with the glomeruli were particulate matter, some fragments of tubules, tubular basement membranes and liberated parietal glomerular capsules. This top layer was pipetted off, diluted with sterile isotonic solution of sodium chloride and centrifuged at 2,000 r.p.m. for three minutes. The glomeruli settled to the bottom. The supernatant solution was saved. The glomeruli were rewashed (three times) with saline solution, and each time the supernatant solution was saved. The resultant sediment, containing minimal nonglomerular cortical contaminants (fig. 1), was made up with saline solution to a volume in which on the average there were 60,100 glomeruli per cubic centimeter. Twelve per cent of these still retained their parietal capsules. It was particularly fortunate that in our

first preparations we obtained so low a percentage of encapsulated glomeruli. Most of our subsequent preparations of glomeruli have contained on the average 40 per cent encapsulated ones.

Preparation of Cortex Freed from Glomeruli.—The material left in the sucrose solution after the glomerular layer was pipetted off was added to the supernatant solutions indicated under the heading of the preparation of glomeruli. This mixture was spun for five minutes in a centrifuge at 2,000 r.p.m. The sediment was resuspended and washed three to five times. It contained fragments of tubules, tubular basement membranes, parietal glomerular capsules, and minimal numbers of glomeruli. Attempts were made to free the fraction of glomeruli by an alternative method. This was basically similar to that described by Solomon and associates [3] except that the prepared cortex was forced through a sieve. Though this procedure yielded an abundance of highly purified glomeruli, it failed to free the non-glomerular fraction of glomeruli. One reason for this is that the isolated glomeruli adhere to the sides of vessels and are not easily eliminated. As a result, the reconstituted suspension of this fraction contained on the average 1,750 glomeruli per cubic centimeter.

The three types of suspension were either used immediately or stored at —25 C. and preserved with sodium ethylmercurithiosalicylate (merthiolate® N.N.R.) 1:10,000. Samples of all these suspensions were cultured aerobically and anaerobically prior to use and were consistently free of bacterial contaminants.

Inoculation of Rabbits for the Preparation of Antiserum.—The individual suspensions were mixed with aluminum hydroxide jelly, which served as an adjuvant.[3] The jelly was prepared by the method of Tracy and Welker.[4] Jelly was added to the suspension until the supernatant solution obtained by centrifugation gave a negative result in the biuret test. Generally, equal parts of suspension and jelly were used. This mixture was liquid enough to pass through a 19 gage needle. An inoculation was made into each thigh of male rabbits weighing approximately 2,500 Gm. After a two week period, inoculations were made with half the amount of material originally injected. A week later the rabbits were bled from the heart. The serum was stored. All serums so collected were tested for the presence of antibodies against dog red blood cells and dog plasma. These tests failed to reveal the presence of such antibodies.

The total amount of material which each rabbit received was as follows:

Whole cortex—9 cc. of the suspension, which was equivalent to 6 Gm. of whole cortex and which contained 1,402 Gm. of lyophilized material and a total of approximately 405,450 glomeruli.

Glomeruli only—6.75 cc. of the suspension and an approximate total of 405,675 glomeruli.

Cortex freed of glomeruli—13.3 cc. of suspension containing 1.402 Gm. of lyophilized material and 23,275 glomeruli.

Inoculation of Dogs.—Healthy dogs fed 1 pound (0.5 Kg.) of pard® daily were kept under observation in metabolism cages for several days prior to inoculation.[4a]

3. Spinka, I., and Weichselbaum, P. K.: Proc. Soc. Exper. Biol. & Med. **38**:447, 1938.

4. Tracy, G., and Welker, W. H.: J. Biol. Chem. **22**:55, 1915.

4a. The ingredients of pard® are: Water, meat by-products, soybean flour, wheat, barley; ground edible bone (2.25 per cent); iodized salt (0.5 per cent); sodium iron pyrophosphate (0.035 per cent); fish liver oil (0.0125 per cent); sodium nitrite (0.007 per cent); irradiated yeast (0.00105 per cent); thiamine (0.001 per cent); nicotinic acid (0.001 per cent); calcium pantothenate (0.001 per cent); magnesium sulfate (0.001 per cent); copper sulfate (0.0004 per cent); pyridoxine (0.0003 per cent); riboflavin (0.0002 per cent).

Intake of water and output of urine were measured. Urine was examined daily for albumin. The sediment was examined microscopically. Blood was withdrawn periodically for serum protein and nonprotein nitrogen determinations. Each dog was inoculated intravenously with 1 cc. of antiserum per pound of body weight. Renal biopsies were made on the seventh to eighth days. A few days later inoculations were made again with the same amount of serum, and the dogs were generally killed from ten to twenty days after the second inoculations. Complete autopsies were made.

Routine sections representing all organs, including many sections from the kidneys, were studied microscopically. Except for those of the kidney, there were no visceral lesions that could be attributed to the antiserum.

RESULTS

The degrees of glomerulonephritis produced in the dogs were classified into three grades. Grade 1 corresponded to minimal proliferative and hypertrophic changes in endothelial and visceral epithelial cells. In

Degree of Albuminuria, Grade of Glomerulonephritis and Blood Chemistry (Average Values) of Dogs Inoculated with Antiserums

Type of Serum	Dogs	Degree of Albuminuria on the Basis of 4+	Grade of Glomerulonephritis	Serum Protein, Gm. per 100 Cc.		Nonprotein Nitrogen, Mg. per 100 Cc.	
				Before Inoc.	After Inoc.	Before Inoc.	After Inoc.
Whole cortex	6	2.7+	2.7	6.45	5.80	35.7	41.5
Glomerular	6	2.5+	2.6	6.30	5.90	36.3	39.5
Cortex without glomeruli	6	0	0	6.4	6.05	36.2	35.5

grade 2 (fig. 2) there were abundant hyperplastic and hypertrophic changes of endothelium and visceral epithelium associated with obliteration of numerous capillary loops. There was some thickening of the basement membrane and mesangium, with minimal exudate in glomerular spaces and relatively few hemorrhagic tubular casts. Grade 3 (fig. 3) differed from grade 2 only in that there was considerable hemorrhagic exudate in glomerular spaces, its presence leading to adhesions and crescent formation. Often pronounced thickening and sometimes apparent splitting of the basement membrane were noted, while hemorrhagic casts were more abundant, and there were extensive areas of tubular atrophy not necessarily associated with the presence of casts.

It was found as a general rule that animals which responded with grade 2 or 3 glomerulonephritis after the first injection of nephrotoxic serum did not react unfavorably to a second inoculation of the serum and grade 2 nephritis was not converted into grade 3. On the other hand, when the reaction was grade 1 or when the kidney was relatively normal, a second inoculation might intensify the process to grade 2 or 3.

Within the limits of observation, rarely exceeding four weeks following the first inoculation, the kidneys at autopsy often showed marked regression of grade 2 lesions, while in grade 3 lesions there was considerable resultant repair with atrophy of glomeruli as well as fibrous adhesions and scarred crescents. The lesions in other instances, however, were still quite active.

It can readily be seen from the table that whole cortex antiserum gave rise to glomerulonephritis with an average grade of 2.7 (4 dogs, grade 3; 1, grade 2 to 3, and 1, grade 1 to 2). The 6 dogs inoculated with glomerular antiserum yielded an average grade of 2; 2 dogs, grade

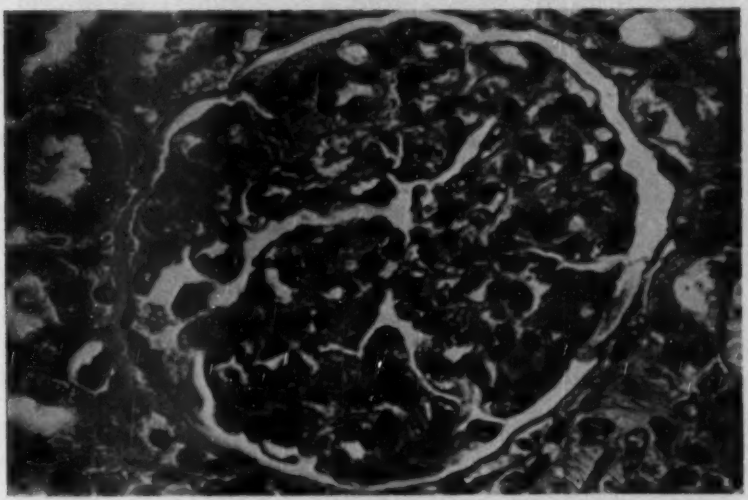

Fig. 2.—Grade 2 glomerulonephritis: proliferation and hypertrophy of endothelial and visceral epithelial cells. × 430.

3; 1, grade 2 to 3; 2, grade 1 to 2, and 1, a subgrade 1. The somewhat lesser reactivity of this serum may be explained by the fact that more glomeruli were broken up in the Waring blendor in the preparation of whole cortex than was anticipated. Since the number of pure glomeruli used for injection was based on the number enumerated in the suspension of whole cortex, it may well be that fewer glomeruli were used as antigen. By contrast with serums obtained by inoculating whole cortex and glomeruli, the serum obtained by the injection of cortex relatively free of glomeruli was totally inactive despite the fact that the amount of lyophilized dried relatively aglomerular cortical material was equivalent to that used for whole cortex.

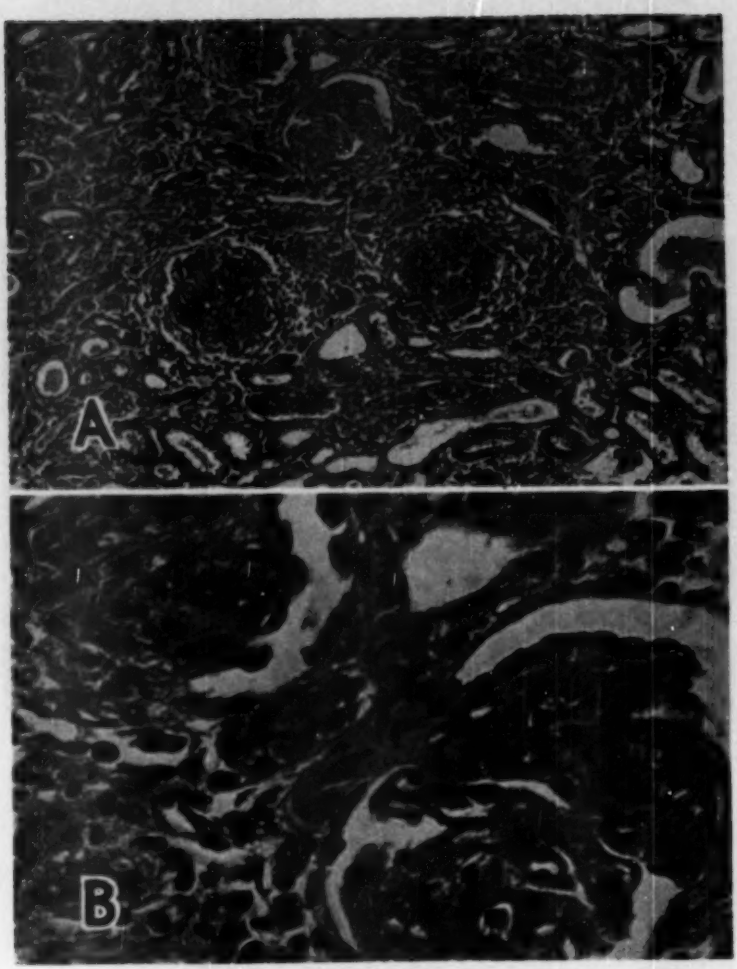

Fig. 3.—*A*, grade 3 glomerulonephritis: capsular crescents and adhesions, tubular casts and tubular atrophy. × 100. *B*, grade 3 glomerulonephritis. High power view of two of the glomeruli illustrated in *A*, showing crescents and adhesions with early sclerosis of one glomerulus. × 430.

SUMMARY

A simple method of separating renal glomeruli from other renal components is described. They can be obtained in large numbers and with a high degree of purity.

With isolated glomeruli being used as antigen, a potent nephrotoxic serum can be obtained, whereas all other cortical components of the kidney, relatively free of glomeruli, are ineffective in the production of such serum.

It seems clear, therefore, that in the production of nephrotoxic antiserums the antigenicity of whole renal cortex lies within the glomerulus.

ACTION OF LYSOZYME ON GASTROINTESTINAL MUCOSA

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LYSOZYME is an enzyme characterized by its ability to effect the lysis of certain bacteria, notably Micrococcus lysodeikticus, which was isolated by Fleming,[1] and Sarcina lutea. The lytic power of lysozyme is attributed to its ability to digest the mucoid capsule of the susceptible organism. Lysozyme is widely distributed in nature, being found in plants as well as in animals. Early studies of this enzyme were confined mainly to the aforementioned lytic action.

The recent investigation of Meyer and his co-workers [2] suggesting a relationship between lysozyme and certain ulcerative diseases of the alimentary tract has aroused widespread interest. These workers reported that the concentration of lysozyme is increased above normal in the pyloric and duodenal mucosa as well as in the gastric juice of patients with peptic ulcer. Patients with ulcerative colitis were found to have extremely high concentrations of lysozyme in the feces.

These investigators also claimed that pure egg-white lysozyme when perfused in high concentration through a Pavlov pouch for several hours caused complete removal of the surface mucus. Other experiments showed that feeding of large amounts of lysozyme led to lesions of the intestine.

The present work was undertaken in order to study the effect which crystalline lysozyme exerts on the gastrointestinal mucosa and the mechanism of its action.

EXPERIMENTAL PROCEDURES AND OBSERVATIONS

Effect of Lysozyme on the Gastric Mucosa of the Rat in Vivo.—Acute experiments were carried out on rats which had been fasted for twenty-four hours and then anesthetized with pentobarbital sodium U. S. P. The abdomen was opened,

From the Department of Clinical Science, University of Illinois College of Medicine.

1. Fleming, A.: Proc. Roy. Soc., London, s.B **93**:306, 1922.

2. (*a*) Meyer, K.; Prudden, J. F.; Lehman, W. L., and Steinberg, A.: Proc. Soc. Exper. Biol. & Med. **65**:220, 1947; (*b*) Am. J. Med. **5**:496, 1948.

and the esophagus was carefully ligated excluding the blood vessels and the vagus nerves. The solution of lysozyme was introduced into the stomach by way of a glass cannula tied in the duodenum 1 cm. below the pylorus. Three cubic centimeters of the solution was allowed to remain in the stomach for one hour and was then replaced by a fresh portion of solution every hour. All gastric tissues were examined grossly and microscopically.

One series of experiments consisted in introducing into rats' stomachs every half hour over a period of four to six hours a solution of pure lysozyme.[3] The concentration of lysozyme was 2 mg. per cubic centimeter. The enzyme was dissolved in McIlvain's buffer solution containing 0.2-molar sodium chloride at p_H 5.3. The same buffer solution, p_H 5.3, without lysozyme was used as a control.

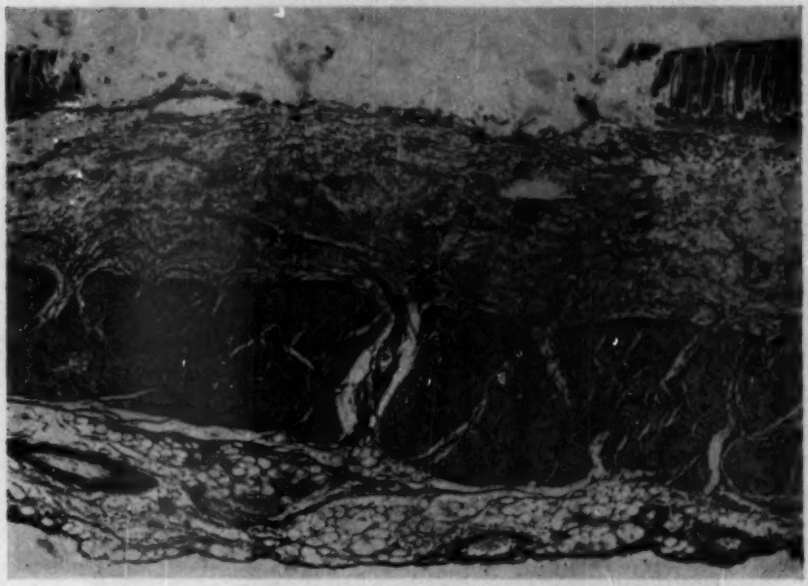

Fig. 1.—Section through one of the ulcers of a rat's stomach which had been perfused for five hours with lysozyme dissolved in McIlvain's buffer solution (p_H 5.3). The ulcer had penetrated beyond the muscularis mucosae. The submucosa is thickened. Note the intact adjacent mucosa. (Mayer's mucicarmine stain for mucus.)

In a second series the same buffer solution with the same concentration lysozyme was introduced into the stomach each half hour over a period of three hours, then 0.2 per cent pepsin in 0.15-normal hydrochloric acid containing 0.2-molar sodium chloride was introduced every half hour over an additional period of three hours. Two sets of control animals were used in this series. One group received the buffer solution alone as a substitute for the lysozyme solution; i. e., this group

3. The lysozyme used in these experiments was crystalline egg-white lysozyme supplied by the Armour Laboratories.

was treated successively with buffer solution and then with acid-pepsin solution. A second control group received saline solution in place of acid-pepsin solution; i. e., this group was treated successively with lysozyme in buffer solution and then with saline solution.

A third series of experiments was carried out by using a solution containing 0.15-normal hydrochloric acid, 0.2 per cent pepsin, 0.2-molar sodium chloride and 2 mg. of lysozyme per cubic centimeter—all in a single solution. Two sets of control animals were also used: One received the lysozyme dissolved in buffer

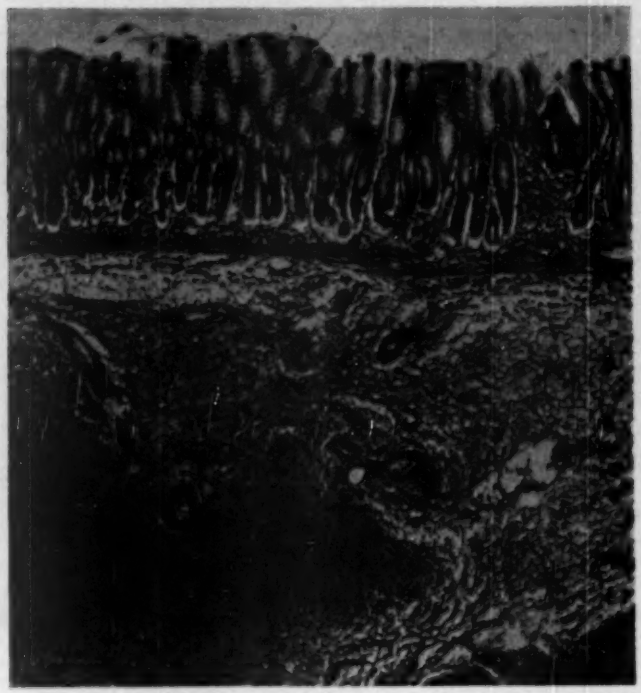

Fig. 2.—Section of a rat's stomach which had been perfused for five hours with lysozyme dissolved in McIlvain's buffer solution (p_H 5.3). Note congestion, hemorrhage and hemolysis in the submucosa. The mucosa is rather normal. (Hematoxylin and eosin stain.)

solution without acid-pepsin solution; the other received the acid-pepsin solution without lysozyme. All perfused solutions were changed every half hour for four to six hours.

Results.—In experiments of the first series, lysozyme dissolved in buffer solution produced gross or microscopic damage in 3 of the 6 rats. In 1 animal an ulcer penetrated the muscularis mucosae (fig. 1). In the remaining 2 the damage consisted of erosions in one and hemorrhage in the other. In a fourth animal, congestion,

hemorrhage and hemolysis were found in the submucosa beneath the normal mucosa (fig. 2). In control animals treated with buffer solution without lysozyme no gross or microscopic changes were found. The results of this series of experiments are tabulated in table 1.

Table 2 contains the results of the second series of experiments. Ulcers were found in the stomachs which had been perfused with lysozyme in buffer solution and then with hydrochloric acid–pepsin solution. They occurred most frequently at the lesser curvature of the pyloric region. In some cases hemorrhage was seen over the whole surface of the gastric mucosa. In a portion of gastric mucosa, after perfusion of these solutions (fig. 3), an area of edema was seen near the surface of the glands, with edema and hemorrhage below. In the control animals treated with buffer solution without lysozyme and then with acid–pepsin solution, no damage was found. In the control animals treated with lysozyme in buffer solution and then with saline solution, slight erosions or hemorrhage occurred. Of 7 rats, erosions were found in 3, surface hemorrhage in 1, and no change in 3.

In the third series of experiments (table 3) damage was found in the stomachs of all animals treated with lysozyme, hydrochloric acid and pepsin in one solution (fig. 4 *B*). Of 6 rats, erosions occurred in 3 and hemorrhage in 1. The micro-

TABLE 1.—*Effect of Lysozyme in Buffer Solution on the Gastric Mucosa of Rats*

Treatment	Rats	Results: Gross Findings: No Change	Hemorrhage	Erosion	Ulcer	Results: Microscopic Observations: Surface Mucosa Normal	Some Surface Cells Destroyed	Loss: Whole Mucosa at Ulcer Portion
Buffer solution with lysozyme (p_H 5.2) (2 mg./cc.)	6	3	1	1	1	3	3	1
Buffer solution (p_H 5.2)	6	6	0	0	0	6	0	0

scopic changes consisted of congestion, hemorrhage and hemolysis. In control animals treated with the same acid–pepsin solution without lysozyme the damage was slight (fig. 4 *A*), consisting of slight erosions in 5 and hemorrhage in 1. The other set of control animals, treated with lysozyme in buffer solution, also showed only slight changes, as illustrated in figure 4 *C*. The damage found after the two enzymes had been perfused together was greater than after one alone. When one was perfused immediately after the other, the damage caused was usually greater than that caused by a mixture of the two enzymes in one solution. This can be seen when the results in tables 2 and 3 are compared.

Effect of Lysozyme on the Mucous Membrane of the Rat's Colon.—Eight rats were fasted for twenty-four hours and then anesthetized with pentobarbital sodium U. S. P.; the abdomen was opened and a glass cannula tied in the ascending colon. After gentle flushing with 5 cc. of tap water to remove remaining feces, the sigmoid colon was tied off. Five cubic centimeters of either lysozyme in buffer solution or buffer solution alone was instilled into the colon; these were removed and replaced with fresh portions at thirty minute intervals. Four rats received lysozyme, 2 mg. per cubic centimeter in buffer solution p_H 5.4, and 4 rats served as controls, receiving buffer solution alone.

Results.—One pair of rats receiving the perfused solutions for four hours showed no damage. The other pairs, treated by perfusion for six to eight hours, showed marked hemorrhage, engorgement and erosions in the animals receiving lysozyme; the control animals showed no lesions. Figure 5 is a photomicrograph

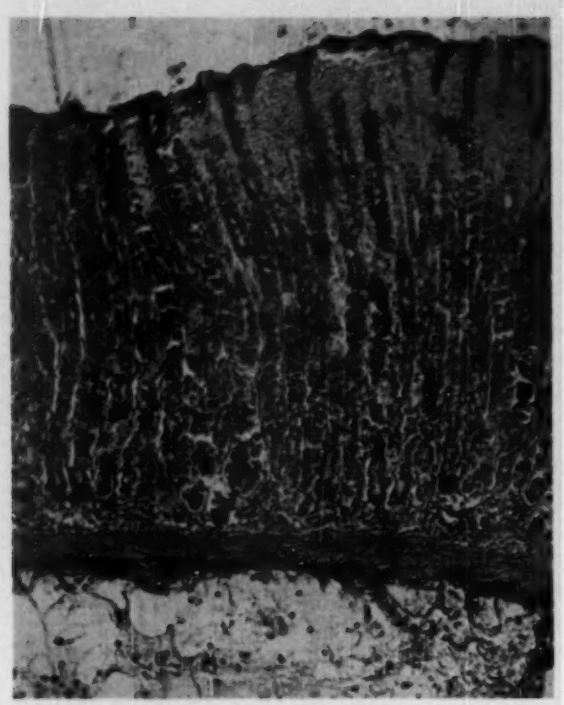

Fig. 3.—Section of a rat's stomach which had been perfused with lysozyme in buffer solution for three hours and then with hydrochloric acid-pepsin solution for three hours. Edema and plasma cell infiltration are present beneath the surface epithelium. Some of the glands are gone, but the lower parts of the glands look normal. (Mayer's mucicarmine stain.)

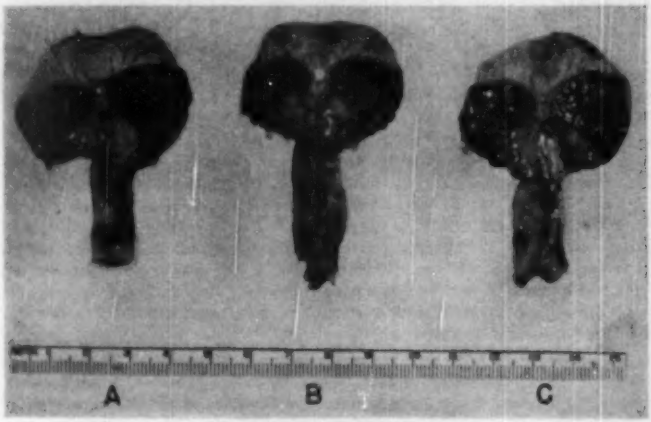

Fig. 4.—Stomachs of rats (series 3): *A*, rat's stomach which was perfused with hydrochloric acid-pepsin solution alone for six hours. There is little damage in the gastric mucosa. *B*, rat's stomach which was perfused with lysozyme dissolved in hydrochloric acid-pepsin solution. Note the severe damage spread over the whole gastric and duodenal mucosa. *C*, rat's stomach which was perfused with lysozyme dissolved in buffer solution (p_H 5.3). Note the several slight erosions in the gastric mucosa and one erosion in the duodenal mucosa.

of the colon of a rat treated with lysozyme. It shows that hemorrhage may involve the submucosa at a time when the overlying mucous membrane is intact and shows no depletion of mucus.

Effect of Lysozyme on Gastric Mucosa of Dog and Cat in Vitro.—Sections prepared by the freezing-drying procedure of Wang and Grossman[4] were used. The slides were run briefly through xylene and alcohol to water. One set of mounted sections, 8 microns thick, was incubated in buffer solution containing lysozyme, 2 mg. per cubic centimeter, at 35 C. for three hours, and was then compared with the other set of sections, incubated in buffer solution without lysozyme. The Hotchkiss[5] method of staining polysaccharide with periodic acid was used for

TABLE 2.—*Effect of Lysozyme Followed by Pepsin–Hydrochloric Acid Treatment*

Treatment		Results							
			Gross Findings				Microscopic Observations		
First 3 Hours	Second 3 Hours	Rats	No Change	Hemorrhage	Erosion	Ulcer	Surface Mucosa Normal	Some Surface Cells Destroyed	Loss: Whole Mucosa at Ulcer Portion
Buffer solution with lysozyme 2 mg./cc. (ps 5.3)	Pepsin (0.2%) 0.15-normal HCl	7	0	0	0	7	0	7	7
Buffer solution (ps 5.3)	Pepsin (0.2%) 0.15-normal HCl	7	7	0	0	0	7	0	0
Buffer solution with lysozyme (ps 5.3)	Saline	7	5	1	2	0	5	2	0

TABLE 3.—*Effect of Hydrochloric Acid, Pepsin and Lysozyme in a Single Solution on the Gastric Mucosa of Rats*

Treatment	Rats	Results							
			Gross Findings				Microscopic Observations		
		No Change	Hemorrhage	Erosion	Ulcer	Surface Mucosa Normal	Some Surface Cells Destroyed	Loss: Whole Mucosa at Ulcer Portion
Buffer solution with lysozyme (2 mg./cc. ps 5.3)	6	4	1	1	0	4	2	0
Acid pepsin (0.2% pepsin in 0.15-normal HCl) + lysozyme	6	0	1	2	3	0	3	3
Acid pepsin (0.2% pepsin in 0.15-normal HCl)	6	0	1	5	0	0	6	0

mucus staining. The two sets of slides were stained simultaneously, with identical treatment.

Results.—Sections taken from the gastric mucosa of 4 dogs and 3 cats and prepared by the freezing-drying method were studied. In figure 6 a section from the gastric mucosa of a dog illustrates the effect of buffer alone (6 *A*) and another the effect of lysozyme in buffer (6 *B*). In the latter most of the mucus within the surface epithelial cells showed vacuolation (fig. 6 *B*); in the buffer control, as shown in figure 6 *A*, no vacuolation occurred. The contrast between the two sections lies not so much in the intensity of the staining of the mucus as in the loss of cellular outline and the loss of homogeneity of the mucus in the section

4. Wang, K. J., and Grossman, M. I.: J. Lab. & Clin. Med. **34**:292, 1949.

5. Hotchkiss, R. D.: Arch. Biochem. **16**:131, 1948.

exposed to lysozyme. Such results could not be demonstrated in the ordinary tissue section which had been fixed with 70 per cent alcohol.

Effect of Lysozyme on Viscosity, Opacity and Microscopic Appearance of Gastric Mucus.—An attempt was made to gain more information about the action of lysozyme on mucus and cells by "in vitro" experiments on gastric mucus. In periods of observation up to six hours in length, lysozyme failed to cause a fall in viscosity in solutions of commercial gastric mucin or in fresh, alkaline, opaque mucus from the cat's stomach when present in concentrations up to 2 mg. per cubic centimeter. Viscosity of the commercial mucin was measured by the Ostwald method, and that of the fresh mucus by a crude falling bead method. When lysozyme was added to clear gastric juice buffered to p_H 6, a white precipitate formed.

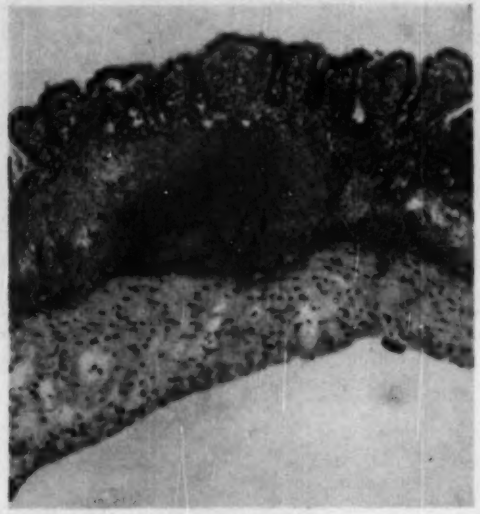

Fig. 5.—Photomicrograph of rat's colon which was perfused with lysozyme solution for eight hours. In the submucosa a large hemorrhage lies beneath the apparently intact epithelium. (Hematoxylin and eosin stain; × 120.)

Surface cells suspended in the opaque, alkaline mucus showed no signs of disintegration in the presence of lysozyme in concentrations of 2 per cent in buffer at p_H 5 to 6. Lysozyme in solution in acid gastric juice did not hasten the rate of disintegration of cell bodies suspended in the mucus above that of the disintegration caused by acid juice alone.

In one series of experiments the cell-containing mucus was left with the lysozyme, which was in concentrations of 0.5, 0.75, 1.5 and 2 per cent in buffer at p_H 6, with toluol as preservative, for three days at room temperature. Smears were then compared with controls in which buffer alone was present. In another series the effect on the gross and the microscopic appearance of the mucus of 1 per cent and 2 per cent lysozyme in buffer of p_H 6, buffer of p_H 5, gastric juice of p_H 6, gastric juice of p_H 1 to 2 and in buffers of p_H 5 and 6 without lysozyme was

studied. Grossly, the mixture containing lysozyme appeared more densely white than the buffer control mixture. Microscopically, the cells in the enzyme mixture stood out more clearly on the slides. This appeared to be due to a deeper yellowish tinge of the cytoplasmic granules, darker outlining of walls and a slight increase in size of cell as compared with the controls. In some cases granular clumps of material, seen in all smears, had a deeper yellowish tinge in the presence of lysozyme than in the controls (buffer alone).

In addition to the tests mentioned, mucus which had been exposed to 1 per cent lysozyme in buffer (p_H 6) for three days was then made alkaline (p_H 7 to 8), and smears were examined at once. No marked change occurred in the cells in two hours, and two days later no definite change could be seen.

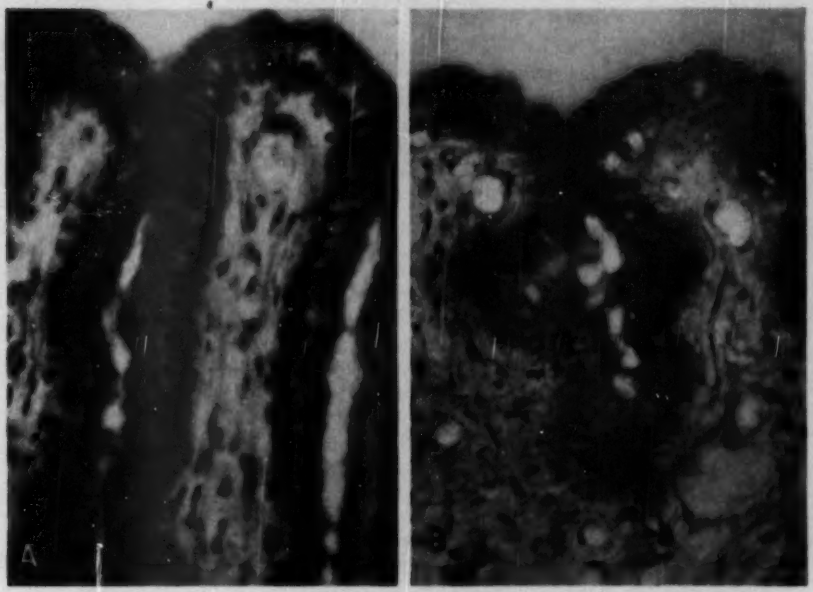

Fig. 6.—*A*, dog's gastric mucosa after being incubated in buffer solution at p_H 5.3 for three hours. The mucus was stained by the Hotchkiss technic. Note that there is no vacuolation in the surface mucoid cells. The mucus in the epithelial cells appears dark. *B*, dog's gastric mucosa after being incubated for three hours with lysozyme (2 mg. per cubic centimeter) dissolved in McIlvain's buffer solution (p_H 5.3). Note the vacuolation in the surface epithelial cells and the mucus in the epithelial cells, which is poorly stained.

Also, mucus in 2 per cent lysozyme in buffer (p_H 6) was incubated for two hours. Then acid-pepsin (1 mg. of pepsin per cubic centimeter of hundredth-normal hydrochloric acid) was added and smears watched. No increase of susceptibility to acid-pepsin digestion could be detected.

In another experiment ranks of surface cells secured by scraping the mucosa were exposed to lysozyme and observed for one hour. No separation of the cells could be seen.

COMMENT

These studies clearly demonstrate that lysozyme is capable of damaging mucous membranes. They give no clue as to how this damage is brought about, and specifically they do not support the conclusion of Meyer and his co-workers [2b] that lysozyme is capable of removing the surface mucus from the mucosa. The evidence that Meyer and co-workers offered in support of this contention is unconvincing because they compared the mucosa of a pouch perfused with lysozyme with the nonperfused mucosa of the main stomach. They offered no controls regarding the pouch before perfusion or the effect of perfusion of buffer without lysozyme.

Observation of the junction of the normal and the damaged parts of the gastric mucosa following treatment with lysozyme shows that there has been no depletion of the mucus in the cells immediately adjacent to the damaged region (see fig. 1). This speaks against the idea that removal or destruction of mucus, rendering the mucosa susceptible to acid-pepsin action, is the mechanism of the injurious action of lysozyme.

It should be kept in mind that the concentrations of lysozyme used in these studies are much higher than those encountered clinically even in ulcerative diseases.

No substrate of lysozyme has yet been demonstrated in the alimentary tract. Meyer, Smith and Palmer [6] originally stated that a mucopolysaccharide fraction isolated from hog gastric mucosa was hydrolyzed when incubated with egg-white lysozyme, yielding reducing sugar, but in a later report [2b] it was stated that the acid and neutral polysaccharides of the hog gastric mucosa were not depolymerized by lysozyme. Our studies also fail to demonstrate the site of action of lysozyme on the gastrointestinal mucosa.

SUMMARY AND CONCLUSIONS

Lysozyme produces erosions and hemorrhages in the gastric and colonic mucosa of rats and increases the injurious action of hydrochloric acid and pepsin in vivo. When sections of dog and cat stomach are incubated in lysozyme, vacuolation of the mucus of the surface epithelial cells and disorganization of both the surface epithelial cells and the neck mucoid cells occur. Lysozyme does not decrease the viscosity of gastric mucus, nor does it cause disintegration or disaggregation of surface epithelial cells suspended in mucus, or render them more susceptible to acid-pepsin action. The mechanism of the injurious action of lysozyme has not been satisfactorily explained.

6. Meyer, K.; Smith, E. M., and Palmer, J. W.: J. Biol. Chem. **119**:73, 1937.

INTESTINAL LIPODYSTROPHY (WHIPPLE'S DISEASE) OCCURRING WITH PARATHYROID HYPERPLASIA AND NEPHROSIS

Report of a Case with Autopsy

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THE CASE of intestinal lipodystrophy which we shall report, though similar in many respects to that described by Whipple [1] in 1907, is distinguished by a hitherto unreported finding—that of concomitant parathyroid hyperplasia and nephrosis.

Since the recent excellent critical review of the literature by Rosen and Rosen [2] in 1947, many other cases of intestinal lipodystrophy have been reported within a short time, and we personally know of several as yet unpublished ones.

In addition to the cases already analyzed by Rosen and Rosen,[2] who apparently overlooked a case reported by Gaertner [3] in 1938, we have found: reported in 1945—by Clemmesen,[4] 1 case; in 1946—by Escudero, Mosto and Landabure,[5] 1 case; in 1947—by Pemberton, Comfort, Fair and Zaslow,[6] 3 cases; by Frei,[7] 1 case (case 2); by Oliver-Pascual, Galan, Oliver-Pascual and Castillo,[8] 1 case; in 1948—by Newman and Pope,[9] 1 case; by Chapnick,[10] 1 case; by Fernández Luna and Latienda,[11]

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This study was sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

1. Whipple, G. H.: Bull. Johns Hopkins Hosp. **18**:382, 1907.
2. Rosen, M. S., and Rosen, S. H.: Am. J. Path. **23**:443, 1947.
3. Gaertner, K.: Frankfurt. Ztschr. f. Path. **52**:529, 1938.
4. Clemmesen, J.: Acta. med. Scandinav. **121**:495, 1945.
5. Escudero, P.; Mosto, D., and Landabure, P.: Rev. Asoc. méd. argent. **60**:795, 1946.
6. Pemberton, J. de J.; Comfort, M. W.; Fair, E., and Zaslow, J.: Surg., Gynec. & Obst. **85**:85, 1947.
7. Frei, R.: Schweiz. Ztschr. f. Path. u. Bakt. **10**:685, 1947.
8. Oliver-Pascual, E.; Galan, J.; Oliver-Pascual, A., and Castillo, E.: Rev. españ. enferm. ap. digest. y nutrición **6**:213, 1947.
9. Newman, B., and Pope, R. H.: Gastroenterology **11**:120, 1948.
10. Chapnick, H. A.: Ann. Int. Med. **29**:549, 1948.
11. Fernández Luna, D., and Latienda, R. I.: Rev. Asoc. méd. argent. **62**:590, 1948.

1 case, in which the patient was a child of 17 months, the youngest on record. Also, Black-Schaffer [12] mentioned he had 4 cases, and Cornell,[13] 1 case.

In evaluating the preceding cases we consider the following criteria as essential: (1) diarrhea, with or without laboratory findings of excess fat in the stools; (2) lipid storage in the lymphatic channels of the mucosa of the small intestine and in the adjacent mesenteric lymph nodes with (3) lipophagic reaction. We discount the 3 cases of Pemberton and associates [6] because they do not conform to these criteria. We accept the case of Oliver-Pascual and associates,[8] although no autopsy was made, because (1) the patient had diarrhea, (2) at laparotomy there was evidence of fat and a granulomatous reaction of the small intestine and (3) the adjacent mesenteric lymph nodes obtained at this time contained foam cells. With the aforementioned criteria in mind, we admit Rosen and Rosen's [2] list of 12 cases, add 8 from the cases enumerated in the foregoing paragraph and 1 of our own, making ours the twenty-first case.

Of the authors who reported the 34 cases in the literature interpreted as instances of intestinal lipodystrophy, of which 5 were surgical, only Blumgart,[14] regarding his second case, stated that all the parathyroid glands were dissected out and were normal. Clemmesen [4] wrote that the "neck organs appeared normal" but did not elaborate his statement. Fairley and Mackie [15] also reported that (case 1) two parathyroid glands were found and dissected out, and appeared normal. These authors' cases are sometimes discussed in reference to Whipple's disease. However, Fairley and Mackie [15] themselves did not entertain the diagnosis of intestinal lipodystrophy, and after reviewing their cases, we concur in their opinion.

It can be remarked that the parathyroid glands were observed and named by Sandström in 1880 and recognized as independent organs by Kohn in 1889. Their function was demonstrated by the work of Gley, of Vassale and Generali, and of Erdheim from 1893 to 1914. During this period, in 1908, MacCallum and Voegtlin [16] showed that parathyroid tetany was due to hypocalcemia. It is not surprising, therefore, to find little mention of these glands in discussions of cases prior to 1920.

Although in several cases of intestinal lipodystrophy there was demonstrable renal impairment, only Whipple's [1] case and our case can be classified as instances of nephrosis. Whipple's description of the

12. Black-Schaffer, B.: Am. J. Path. **24**:677, 1948.

13. Cornell, V. H., in discussion on Black-Schaffer.[12]

14. Blumgart, H. L.: Arch. Int. Med. **32**:113, 1923.

15. Fairley, N. H., and Mackie, F. P.: Brit. M. J. **1**:375, 1937.

16. MacCallum, W. G., and Voegtlin, C.: Bull. Johns Hopkins Hosp. **19**:91, 1908.

kidneys in 1907 conforms to the present accepted classification of nephrosis. In 1905 Mueller suggested the term "nephrosis" for the degenerative types of renal disease, but these could not be distinguished clinically from the inflammatory types. Somewhat later, Volhard and Fahr, in 1914, wrote the now classic monograph giving the clinical and anatomic descriptions which differentiated the degenerative from the inflammatory forms of renal disease. Parathyroid hyperplasia occurring with a renal impairment called "nephritis" was reported by MacCallum [17] in 1905.

Comparison of Recorded Cases Regarding Serum Calcium and Phosphorus Levels and Dextrose Tolerance

Author	Serum Calcium, Mg./100 Cc.	Serum Phosphorus, Mg./100 Cc.	Dextrose Tolerance Curve
Amsterdam, H. J., and Grayzel, D. M.: Am. J. M. Sc. 210:666, 1945	Normal	...	.....
Blumgart [14] (case 2)	Tetany	...	.....
Blumgart [14] (case 3)	Tetany, 5.3	9.6	.....
Chapnick [15]	8.7	2.8	Low, flat
Clemmesen [4]	9.3	3.8	Elevated (2.33 hr.)
Escudero and co-workers [5]	7.3	...	.....
Fernández Luna and Latienda [11] (child)	6.0	2.3	.....
Jarcho, S.: Bull. Johns Hopkins Hosp. 59: 275, 1936	.....	...	Low, flat
Newman and Pope [9]	9.0	Normal	.....
Oliver-Pascual and co-workers [8]	.....	...	Low, flat
Pearse [12]	9.1	4.9	Normal
Pemberton and co-workers [6] (case 1)	.....	...	Normal
Saller, B., and McGann, R. J.: Am. J. Digest. Dis. 9:55, 1942	.....	...	Low, flat
Odessky and Burdison, this issue	11.8	4.9	Elevated, prolonged (2.0 hr.)

Furthermore, our case is also distinguished from others in the literature by a high level of serum calcium, associated with a serum phosphorus level within the upper limit of normal, and an elevated, prolonged dextrose tolerance curve (table).

REPORT OF CASE

Present Illness.—A 48 year old white man was admitted to the hospital on Nov. 26, 1948, with the complaint of diarrhea of three weeks' duration, occurring first at night and, later, both day and night. He was feeling well until sixteen months prior to hospitalization, when he began to get tired and could not stand on his feet for any great length of time because of rheumatic-like pain in his legs. Shortly after this he started to have pain across the small of his back. One year prior to admission, there occurred daily vomiting spells, regularly after breakfast and on a few occasions after supper. During this year he lost 40 pounds (18 Kg.) in weight, but his appetite remained good. His diet consisted of

17. MacCallum, W. G.: Bull. Johns Hopkins Hosp. 16:87, 1905.

such usual foods as cereals, eggs, meats, fruits and vegetables. By March 1948 he felt tired, weak, sleepy and, after walking one block, so short of breath that he stopped work. Constipation developed at this time; nocturia with frequency of urination, two months later. No dizziness or fainting spells were reported. In October 1948 he visited the outpatient clinic complaining of flatulence, for which he was given brewers' yeast tablets. A few days later diarrhea developed, and, therefore, he stopped taking the yeast tablets. The patient continued to have diarrhea, evacuating semisolid, slate-colored stools without abdominal pain, nausea or vomiting. Since the onset of this diarrhea, anorexia had developed, and he had eaten only soups, liquids and gruels.

Past History.—The patient had the usual childhood diseases. He had dementia precox, hebephrenic type, in remission. He was discharged from the Navy in 1944, after twenty months of service, because he could not physically perform his duties. Ten months were served in Alaska, none in the tropics. While in Alaska he had a bout of diarrhea which lasted for a week. He was treated by hemorrhoidectomy in 1939 and by open reduction of a fracture of the left acromial process in 1946; he had chronic bilateral otitis media. His record noted myocardial damage with premature contractions prior to November 1947. An examination for pension purposes in May 1948 disclosed the following: weight, 165 pounds (75 Kg.); pain in the left wrist, hip and ankle; slight swelling, tenderness and limitation of motion of the left wrist and ankle; incomplete bundle branch block since previous electrocardiographic examination. There was no history of exposure to any occupational toxic substances. He admitted drinking beer occasionally but no "hard liquor."

Family History.—His mother had diabetes.

Physical Examination on Admission.—The patient was well developed, poorly nourished, 70 inches (178 cm.) in height, 129 pounds (58.5 Kg.) in weight and appeared chronically ill. He was well oriented and in good contact, but he had a peculiar stare. His pupils did not react to light and they reacted poorly in accommodation. He had full upper and lower dentures, which he did not wear. The heart and lungs were normal. The blood pressure was 110 systolic and 78 diastolic. The abdomen was enlarged and tympanitic throughout. There was slight generalized tenderness in the right upper quadrant of the abdomen. The liver was enlarged to palpation, but there were no other palpable masses. Small anal tags were present. The left shoulder showed a large scar with some deformity of the acromion and a restriction in elevation of the left arm beyond the shoulder.

Laboratory Findings.—On admission, November 26: The Kahn test of the blood and urinalysis gave negative results; the erythrocyte count was 3,810,000 per cubic millimeter; the hemoglobin content was 10.6 Gm. per hundred cubic centimeters (100 per cent = 15.6 Gm.); the leukocyte count was 11,700 per cubic millimeter, with a normal differential count. Chemical examination of the blood showed: total plasma protein, 5.6 Gm. per hundred cubic centimeters, with 3.7 Gm. of albumin, 1.8 Gm. of globulin and an albumin-globulin ratio of 2; the cephalin flocculation test was negative; the icteric index was 4. Subsequent laboratory data: December 10 the sulfobromophthalein sodium test showed no retention at the end of forty-five minutes; the prothrombin time was normal; the basal metabolic rate was plus 9. December 16 an analysis of the gastric content showed a total acidity of 14 degrees with a free hydrochloric acid of 8 degrees; after injection of histamine the total acidity was 48.6 degrees and the free hydrochloric acid 38.4 degrees. On December 29 an erythrocyte count was 4,470,000 per cubic millimeter; the hemoglobin content was 10.0 Gm. per hundred cubic centimeters; the leukocyte count was 14,250 per cubic millimeter, with a normal differential count;

the packed cell volume was 49 per cent; the sedimentation rate was within normal limits. The anemia was macrocytic and hypochromic. December 31 the serum calcium was 11.8 mg., and the phosphorus was 4.0 mg. per hundred cubic centimeters; blood serum lipase was 0.05 cc.; the dextrose tolerance curve was as follows:

	Mg. per 100 Cc.
Fasting	100
0.5 hr.	119
1 hr.	150
1.5 hr.	170
2 hr.	166
3 hr.	160

No glycosuria was present during this test. Smears for malarial infection were negative on several occasions. Repeated examinations of the stools did not show occult blood, parasites or pathogenic organisms on culture. The stools were gray and semisolid, contained fatty acid crystals and with sudan III showed a marked increase of free fat. On Jan. 10, 1949, three hours post mortem: Nonprotein nitrogen was 190 mg. per hundred cubic centimeters; pneumococci were isolated from the spleen in pure culture.

Roentgenologic Findings.—In April 1948 there were: minimal hypertrophic arthritic changes of the vertebral bodies of the sacroiliac joint; scoliosis with convexity to the right of the dorsal spine; no osteoporosis of the vertebrae or of the left femur (the right femur was not examined); a small fenestral defect of the ninth rib. On November 30 a routine roentgenogram of kidneys, ureters, and bladder showed somewhat enlarged kidneys but no demonstrable abnormal calcareous deposits. In December, on fluoroscopic examination, the left side of the diaphragm was slightly higher than the right, but the heart and lungs appeared normal. Roentgenograms after a barium sulfate enema showed no abnormalities. A gastrointestinal series demonstrated a normal esophagus and stomach, a rather large and hypomotile duodenal bulb, an accentuated rugal pattern of the second and third portions of the duodenum and the proximal part of the jejunum with the contained barium sulfate appearing in blotches and adherent to the rather wide-appearing mucosal folds, which measured 4 to 7 mm. in height. There was no evidence of obstruction.

Electrocardiograms.—Tracings on three occasions during the hospitalization of the patient showed severe myocardial damage with incomplete bundle branch block.

Hospital Course.—The patient was treated symptomatically, without improvement. He made no complaints except of frequent stools, which were greasy. He kept losing weight and became very weak. Several fainting spells, lasting less than one minute, were recorded, and on one such occasion it was noted that he was completely relaxed during the blackout and that his muscles twitched a little during recovery. December 23 he felt a sudden pain in the right shoulder. Impaired breath sounds were heard at the base of the right lung on Jan. 5, 1949. Subsequently, he was given two blood transfusions and several intravenous infusions of a protein hydrolysate (amigen® N. N. R.) and dextrose. January 8 he complained of pain in the lower left side of the chest, and his temperature, which up to this time had been subnormal, rose to 101 F. rectally. A roentgenogram of the chest showed only an elevation of the left side of the diaphragm. He declined rapidly in spite of all supportive measures and died on January 10.

Necropsy (three hours after death).—The body, which weighed 115 pounds (52 Kg.), was that of a well developed, clearly emaciated white man. The skin was sallow and the mucous membranes were pale. The abdominal wall contained atrophic fat and a moist, bright, reddish brown musculature. There was no fluid in the peritoneal cavity. The serosa was smooth and glistening, but that of the anterior surface of the stomach was slightly roughened and shaggy. The organs were in their normal relationship. The liver extended 3 cm. below the costal margin in the midclavicular line and 9 cm. below the xiphoid process. The gallbladder was normally distended. There was no fluid in the pleural cavities. The right lung was free. The left lung was partially covered with a fibrinous exudate and contained diffuse dense fibrous adhesions. The pleura of both lungs and the pericardium were covered with numerous petechial hemorrhages. The pericardium was irregularly adherent by dense fibrous bands to the heart, and the apposing surfaces were roughened and shaggy. There was a small amount of straw-colored fluid in the sac.

Thyroid Gland: This organ was normal.

Parathyroid Glands: Three were dissected out. The fourth was not found. The right inferior one was smooth, glistening and light brown and measured 1.2 by 0.4 by 0.2 cm. The others were normal.

Larynx: This organ was normal.

Trachea and Bronchi: The mucosa was congested. The tracheobronchial lymph nodes were discrete and soft gray-black on section, the largest measuring 1.5 cm.

Lungs: The apex of the right lung contained a 2.5 cm. puckered scar. The upper and middle lobes appeared normal; the lower lobe was congested. The left lung was bulky, and its pleura was thickened. With the exception of the apical portion, the lung was firm. The cut surface of the upper lobe was edematous and congested; that of the lower lobe was pinkish gray, finely granular and dry.

Heart: The weight was 380 Gm. The surface was shaggy and purplish red. The chambers were of normal capacity. The foramen ovale was patent but functionally closed by an overlapping of the valve. All measurements were within normal limits. A pinkish gray granular vegetation, 6 mm. in its greatest dimension, adhered to the noncoronary aortic cusp at the line of closure. Other leaflets showed mild granular thickening. Cusps were partially fused at the commissure by a similar vegetation, 2 to 3 mm. in diameter. Scattered through the myocardium were yellowish to grayish white scars. There was marked arteriosclerosis with calcification of the coronary arteries and of the root of the aorta but no occlusion.

Spleen: The organ weighed 220 Gm. and was somewhat softened. The capsule was purplish gray and studded with numerous grayish white opaque subcapsular foci, 1 to 2 mm. in diameter. The cut surface was purplish red and the pulp scraped away.

Gastrointestinal Tract: The esophagus appeared normal. Boluses of greenish white pasty material were found in the stomach and small intestine, the mucosa of which was bright and reddish gray. The hyperemia increased in intensity toward the terminal portion of the ileum. The pale, grayish tan mucosa of the large intestine was mottled with green, the mottling being most marked in the descending colon. The rectum appeared normal.

Liver: The weight was 1,600 Gm. The capsule was translucent and covered with opaque areas similar to those of the spleen. The cut surface was reddish tan, and the periphery of the lobules was slightly accentuated.

Gallbladder: The organ was thin and distended with 50 cc. of thick, dark green bile. The mucosa was velvety. The extrahepatic bile ducts were patent.

Pancreas: The organ weighed 75 Gm. and appeared normal.

Adrenal Glands: Together they weighed 12 Gm. They were of average firmness. On section the markings were distinct. The outer cortical strip was pale grayish yellow and the medulla grayish tan.

Kidneys: The right weighed 255 Gm.; the left, 240 Gm. The capsule stripped easily from a smooth surface. On section the cut surface was swollen and pale. The markings were well preserved. A few cortical cysts were present, the largest measuring 7 mm. in diameter. The pelves and the ureters appeared normal.

Bladder, Prostate and Testes: All these were normal.

Bone: The marrow of a lumbar vertebra was moist and reddish brown.

Pituitary Gland: This appeared normal.

Brain: The organ weighed 1,400 Gm. The meninges and brain were slightly edematous, and the vessels were moderately injected. The sulci were slightly widened, but the cortex and the white matter showed nothing of note. On section the left lateral ventricle, the third ventricle and the aqueduct of Sylvius were slightly dilated. The right ventricle and the foramens were normal. The structures at the base of the brain were intact.

Microscopic Examination.—Lungs: In the left lung, large sections of alveoli were filled with blood and an acute inflammatory exudate, predominantly polymorphonuclear leukocytes. Many arterioles within this region were filled with fresh thrombi. Adjoining alveoli were distended with serum. In the right lung were patches of emphysema and partial atelectasis. Clumps of alveolar phagocytes were conspicuous throughout the field.

Heart: Diffuse areas of fibrosis pervaded the myocardium of the left ventricle. Beneath the endocardium were wide bands of acellular collagenous fibers and a few patches of chronic granulation tissue. Many of the muscle fibers were hypertrophied. The epicardial fat was well preserved, but the peripheral portion contained a wide band of fibrous connective tissue with many small hemorrhages. The engorged, thickened capillaries of the chronic granulation tissue extended into the endocardium. The aortic leaflet was irregularly thickened by loose granulomatous tissue in some places and by organizing thrombi in other areas. Within these zones were collections of large mononuclear cells, with abundant cytoplasm and vesicular nuclei, resembling Aschoff bodies. A few polymorphonuclear leukocytes and lymphocytes were also seen. The aorta disclosed thickening, hyalinization, deposition of cholesterol, ulceration and calcification of the intima, the latter two processes involving part of the media. Beyond this were focal collections of lymphocytes, small hemorrhages and clumps of brown pigment.

Spleen: The pulp was congested. Some of the lymphoid follicles were hyperplastic and others disclosed central fibrosis with hyalinization. The intima of the arterioles was thickened and hyalinized. The capsule was irregularly thickened by an increase of fibrous connective tissue which was lined by a single layer of cuboidal epithelium.

Gastrointestinal Tract: Sections through various levels disclosed numerous small hemorrhages in the peripheral portion of the mucosa of the stomach and small intestine. The mucosa of the small intestine was thickened, and the villi were blunted and club shaped, owing to a collection of closely packed foam cells with small nuclei lying within the stroma, but the glands remained intact (fig. 1). A few of these cells were seen within the submucosa and some within distended endothelium-lined spaces. With sudan III stain, fat vacuoles were demonstrated within many of the foam cells and endothelium-lined spaces of the mucosa and the submucosa. Other foam cells stained blue with nile blue sulfate. In some sections the loose stroma of the submucosa was replaced by a granulomatous reaction

composed of dense cellular connective tissue. The muscle coats and the serosa appeared normal. No pathologic changes were evident in the rest of the gastrointestinal tract.

Lymph Nodes: Several mesenteric and periaortic lymph nodes were examined. The mesenteric nodes contained foam cells similar to those in the intestinal mucosa. The cytoplasm of some of these cells was definitely distended with lipoid vacuoles, but no multinucleated giant cells were seen (fig. 2). The reticuloendothelial stroma

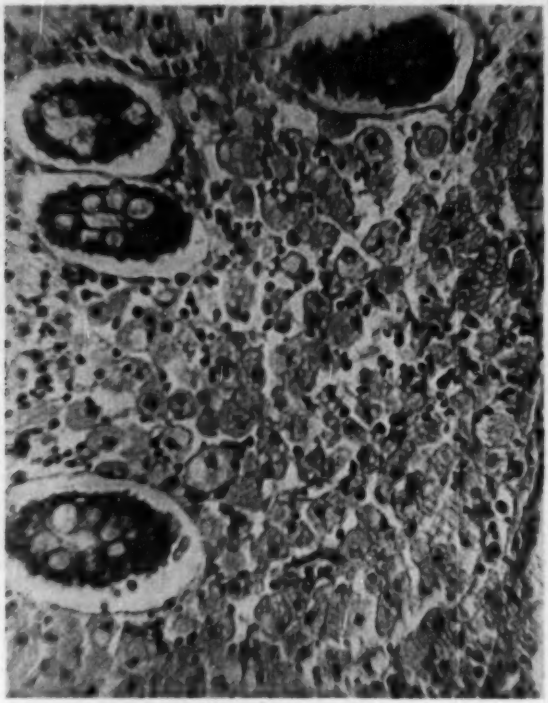

Fig. 1.—Small intestine: Lipophages are accumulating in the mucosa. × 258.

of a few of the mesenteric and periaortic nodes showed patchy areas of fibrosis with hyalinization. Clusters of subcapsular endothelium-lined spaces were filled with clear refractile material.

Liver: The lobules were well preserved, and the peripheries of some of them contained small clusters of fat vacuoles. The central veins were distended with serum. The portal spaces were normal. The capsule was irregularly thickened by finger-like projections of fibrous tissue which corresponded to the opaque areas seen grossly. These were lined by a single layer of cuboidal epithelium which

extended over the entire liver surface. Within these projections were dilated, blood-filled capillaries, diffuse collections of lymphocytes and an occasional histiocyte. Some villi contained small hemorrhages.

Pancreas: The only change noted was a slight diffuse increase of the interstitial connective tissue.

Adrenal Glands: The fascicular zone was hyperplastic. The cytoplasm of large areas of cortical cells stained a deep pink. Vacuolation was less obvious than it usually is. The medulla was abundant but stained poorly. The periadrenal

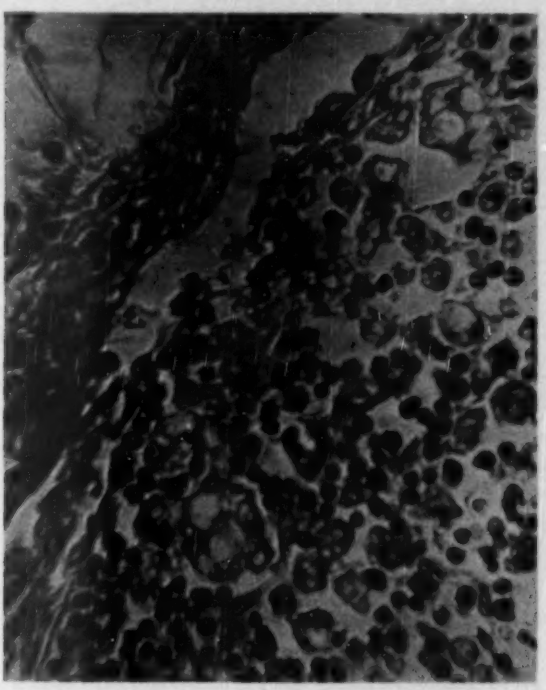

Fig. 2.—Mesenteric lymph node: Lipophages may be seen in the stroma. × 430.

vessels were thickened. On the right side the surrounding fat was lobulated by interlacing fibrous bands extending from the capsule, and on the left side it contained small hemorrhages.

Kidneys: Glomeruli were prominent. The capillaries were patent but empty, and their basement membranes were slightly thickened. The capsules of the glomeruli were thin and irregular, and Bowman's spaces were dilated and contained a pink granular precipitate. The tubules of the cortex were closely packed, and their lumens were filled with a foamy granular precipitate. The epithelium of the convoluted tubules was granular, indistinct and disrupted. The epithelium of most

of the tubules within the cortex, and to a lesser extent within the medulla, was extensively vacuolated. The vacuoles varied in size, some of them distending the cells, leaving only a thin rim of cytoplasm (fig. 3). Sudan III stain revealed no fat. Infrequent hyaline casts were seen in the collecting tubules. The interstitial connective tissue was free of edema and cellular infiltrate. No calcium deposits were found. A small serous cyst lined by a single layer of cuboidal to flattened epithelium was present at the corticomedullary junction.

Bladder: Submucosal vessels were congested.

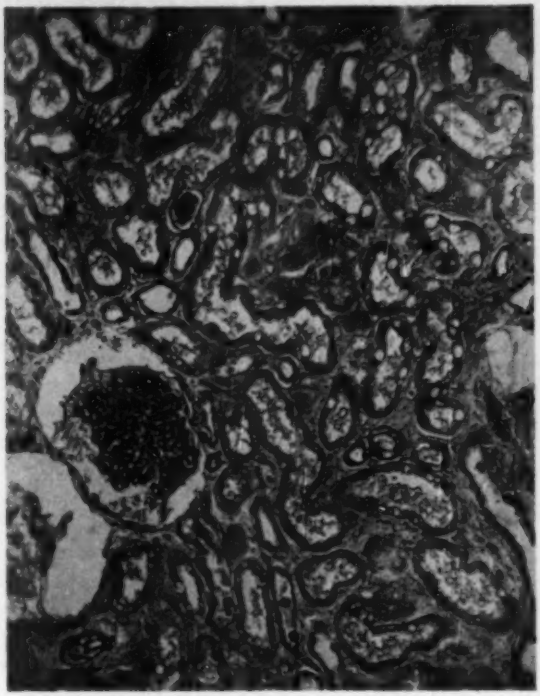

Fig. 3.—Kidney: There is abundant vacuolation of the tubular epithelium. × 100.

Prostate Gland: The gland was hyperplastic.

Testes: The interstitial tissue was slightly edematous and contained a few thickened arterioles.

Thyroid Gland: The gland showed some atrophy and interstitial edema.

Parathyroid Glands: The cells were closely packed. These consisted of scanty, clear cytoplasm and small, round nuclei. There were scattered groups of water-clear cells with abundant cytoplasm and occasional clumps of cells with abundant, deeply acidophilic cytoplasm. The stroma was scanty and contained few fat cells.

Pituitary Gland: The gland was cellular, with the basophilic type predominating. Many of the cells were vacuolated and many of the acini contained colloid.

Pineal Gland: No pathologic changes were observed.

Brain: The only change of note was edema of the cortex and the meninges.

Bone: The marrow from a lumbar vertebra disclosed hyperplasia of the hemopoietic elements, a decrease in the number of megakaryocytes and numerous clumps of brown pigment, largely intracellular.

Anatomic Diagnoses.—Intestinal lipodystrophy (Whipple's disease); chronic vegetative endocarditis of the aortic valve; old healed infarct of the myocardium; chronic adhesive pericarditis; petechial hemorrhages of the pericardium, the pleura and the periadrenal fat; severe generalized arteriosclerosis of the aorta and of the coronary, cerebral and renal arteries; pneumococcic pneumonia of the left lung; fibrous pleural adhesions; chronic passive congestion of the viscera; chronic diffuse perihepatitis and perisplenitis with healing; bilateral nephrosis; edema of the brain; hyperplasia of the right inferior parathyroid gland; hyperplasia of the bone marrow.

COMMENT

"Whipple's disease" is largely a designation of a condition that includes clinical and pathologic evidence of a derangement of the normal process of fat absorption, the precipitating cause of which is as yet unknown. Apparently, there is an anomaly of the mechanism by which fat is passed from the small intestine to the mesenteric lymphatic channels and the portal vein, the precise nature of which is not understood.

In the process of fat metabolism a number of factors are now known to be necessary: lipase; normal electrolyte balance, regulated in part by adrenal cortical hormone; bile; phosphorylation; proper range of hydrogen ion concentration; possibly one or several vitamins; possibly choline; possibly enzyme systems other than lipase, and perhaps other factors. These play an important role in emulsifying, hydrolyzing, dispersing, resynthesizing and phosphorylating ingested fats (Frazer[18]). When one or more of these processes is disrupted, gastrointestinal disturbances may conceivably result. Thus, the symptoms of Pearse's[19] patient were ameliorated on the administration of bile salts. Our patient, however, had a normal flow of bile, as evidenced at autopsy by the distended gallbladder, which contained 50 cc. of bile, and the bile-stained boluses present in the intestine.

The accumulation of fat in the lymph nodes is not limited to Whipple's disease; other ailments, such as mesenteric chyladenectasis, lymphoma, Hodgkin's disease, tuberculosis, Gaucher's disease and primary or metastatic tumors, may show it. Histologically, Whipple's disease differs from the aforenamed conditions in that fat and lipophagic cells also aggregate in the intestinal mucosa. Since foamy macrophages are not normally found in the intestines, it may be assumed that they transport the fat from the intestinal epithelium to the mesenteric

18. Frazer, A. C.: Physiol. Rev. **26**:103, 1946; Brit. M. J. **2**:641, 1947.
19. Pearse, H. E.: Surgery **11**:906, 1942.

lymphatic vessels. This action may be caused by (1) pathologic alteration of the intestinal epithelium, (2) faulty absorption of fat or (3) obstruction of the lymphatic channels.

Infection may be the primary etiologic factor, as suggested by the case histories, two thirds of which record arthritis, rheumatism, pains of joints, rheumatic fever, vegetative endocarditis or positive serologic tests, and the proportion may be even higher, since some histories are incomplete.

Fat accumulating in the tissue spaces may provoke a granulomatous reaction accompanied by multinucleated giant cells. Our sections did not show giant cells, and this may have been due to a nonrepresentative sampling of the lymph nodes or to the fact that the lymphatic channels of the lymph nodes did not show excessive distention and rupture, and, consequently, no foreign body reaction could be expected. Whipple [20] expressed the belief that the changes in the mesenteric lymph nodes are obviously in a much earlier stage than in his case. It is interesting to note that in the case of Apperly and Copley [21] giant cells were demonstrated not only in the mesenteric lymph nodes but also in the kidneys, the liver and the lungs.

The causes of the changes in the kidneys and the parathyroid gland cannot be determined precisely, and it is a matter of conjecture as to which organ was affected first. In cachexia with poor intestinal absorption and with steatorrhea in which an excessive amount of calcium is likely to be excreted in the form of insoluble calcium salts of fatty acids, a serum calcium of 11.8 mg. per hundred cubic centimeters is high. The value for a normal adult is generally between 9 and 10 mg., and a value over 11.5 mg. is regarded as definitely elevated, especially if the plasma proteins are low (Bodansky [22]). Such an increase may be considered presumptive evidence of hyperparathyroidism (Jaffe [23]), in which case a low level of serum phosphorus (normal range, 3.2 ± 0.5 mg. per hundred cubic centimeters, according to Albright and Reifenstein [24a]) would be expected. The 4.0 mg. level of serum phosphorus may be the result of phosphate retention caused by renal damage. Experimentally, Pappenheimer [25] showed that a loss of functional renal tissue results in hyperparathyroidism proportional in volume to the severity of renal damage. Since our patient had a moderate degree of nephrosis, as characterized

20. Whipple, G. H.: Personal communication to the authors.

21. Apperly, F. L., and Copley, E. L.: Gastroenterology **1**:461, 1943.

22. Bodansky, O.: Personal communication to the authors.

23. Jaffe, H. L.: S. Clin. North America **22**:621, 1942.

24. Albright, F., and Reifenstein, E. C.: (*a*) The Parathyroid Glands and Metabolic Bone Disease, Baltimore, Williams & Wilkins Company, 1948, p. 4; (*b*) ibid., p. 59.

25. Pappenheimer, A. M.: J. Exper. Med. **64**:965, 1936.

by abundant vacuolation of the tubular epithelium, this would explain the enlargement of one parathyroid gland. The hyperfunctioning of this gland did not cause skeletal demineralization. Albright and Reifenstein [26b] stated that hyperparathyroidism can occur without bone disease, with definite renal impairment and without demonstrable nephrocalcinosis.

From the foregoing discussion we may infer that the disturbance of fat metabolism could have been responsible for the parathyroid hyperplasia, which could have set off a chain of events leading to renal impairment. Conversely, an infection such as that evidenced by the vegetative endocarditis could have caused renal dysfunction with secondary hyperplasia of one parathyroid gland.

The pneumonia could have contributed to the elevation of the nonprotein nitrogen. We do not believe that the administration of the protein hydrolysate was responsible, because we have roentgenographic evidence to prove that the kidneys were enlarged and therefore impaired before the onset of pneumonia and the administration of the protein hydrolysate.

Notwithstanding a defect of the mechanism of fat absorption and, possibly, protein absorption, the prolonged elevated dextrose tolerance curve without fasting hypoglycemia indicated that our patient absorbed dextrose into the blood stream but deposited it slowly in the tissues because of a deficiency of many dietary elements, a hormonal imbalance or because of his schizophrenia. Clemmesen [4] also reported a nondiabetic type of elevated dextrose tolerance curve without fasting hypoglycemia. This is unlike the low, flat dextrose tolerance curves with hypoglycemia which are frequently found in cases of idiopathic steatorrhea, sprue and celiac disease.

Several writers reported low serum calcium values with tetany. The short episode of tetanus observed in our patient could have been the result of increased cell concentration of phosphate with glycogen breakdown (Cori [26]), uremia, hypoglycemia, drugs or other factors.

In addition, our patient showed many of the well known symptoms secondary to a state of malnutrition: loss of weight, subnormal temperature, diarrhea, low blood pressure and anemia. The anemia, which was macrocytic and hypochromic, has also been encountered in esophageal stenosis, gastric resection, gastroenterostomy, intestinal stenosis, chronic diarrhea as in dysentery, sprue, idiopathic steatorrhea and parasitic infestation (Bodansky and Bodansky [27]).

26. Cori, C. F.: Biological Symposia, Lancaster, Pa., The Jaques Cattell Press, 1941, vol. 5, p. 133.

27. Bodansky, M., and Bodansky, O.: Biochemistry of Disease, New York, The Macmillan Company, 1940, p. 25.

SUMMARY

We have reported a case of intestinal lipodystrophy which is similar in many respects to that originally described by Whipple in 1907. This case is different from the others recorded in the literature in that it presented hyperplasia of one parathyroid gland, nephrosis, a high level of serum calcium, a level of serum phosphorus within the upper limit of normal, a prolonged elevated dextrose tolerance curve and macrocytic, hypochromic anemia.

CRYPTOCOCCOSIS OF THE CENTRAL NERVOUS SYSTEM AND INCIDENTAL CRYPTOCOCCIC GRANULOMA

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CRYPTOCOCCUS histolytica (or torula histolytica) infection of the central nervous system usually results in a fatal meningoencephalytic process, as is well known. The incidental finding of encephalitic cysts due to the action of this mycotic organism has not been recorded in the literature. The present article includes a report of 3 such cases in conjunction with a discussion of 2 others which demonstrate the usual features of cryptococcosis (torulosis) of the central nervous system.

Frothingham,[1] in 1902, first described mammalian C. histolytica infection as it occurred in a horse. His examination was limited to the lungs, where a huge myxomatous tumor was encountered in the posterior portion of the caudal lobe. In this instance the etiologic organism was verified by culture and animal inoculation. Fourteen years later, Stoddard and Cutler,[2] in reviewing the literature on cases of human mycotic infection, ascribed 4 to C. histolytica. They accredited Rush with having reported the first 2 recorded cases of cryptococcosis of the human central nervous system, under the title of "Systemic Oidiomycosis." However, his work was antedated in Germany by the case reports of von Hansemann,[3] in 1905, and Turch, in 1907. Stoddard and Cutler themselves discussed 2 additional cases, described the clinical features of the disease, the morphologic aspects of the organism, its cultural characteristics and pathogenicity for laboratory animals and further clarified the differential features between blastomycosis or oidiomycosis, coccidioidal granuloma and C. histolytica infection. These

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1. Frothingham, L.: J. M. Research **8**:31, 1902.

2. Stoddard, J., and Cutler, E.: Torula Infection in Man, Monograph no. 6, New York, Rockefeller Institute of Medical Research, 1916.

3. von Hansemann: Verhandl. d. deutsch. path. Gesellsch. **9**:21, 1906.

authors proposed the term "histolytica" as descriptive of the tissue response to the organism. By 1931, Freeman [4] was able to collect reports of 43 cases of cryptococcosis of the human central nervous system. Further, he subclassified the organisms according to size, grouping those with diameters between 3 and 5 microns as type I, those with measurements of about 15 microns as type II and a larger form, with diameters from 15 to 40 microns, as type III. He also catalogued the pathologic processes in the central nervous system as (1) a purely meningeal form, (2) a perivascular form, with small granulomatous or cystic lesions superficially localized in the cerebral parenchyma about blood vessels, and (3) an embolic form, with deep-seated lesions in the basal ganglions and the cerebellum. The largest, or type III, organism he found only in association with pure meningeal lesions. The literature was again comprehensively reviewed by Levin,[5] in 1937. His addition of a report of 2 cases to the expanding literature brought the number of reported cases to 60. In 1940, Binford [6] added 14 cases from the literature and his own experience. Voyles and Beck [7] extended the series to cover 108 cases reported through May 1946. Since this date Mezey and Fowler,[8] Kraimer and associates [9] and Mider and associates [10] have reported single cases. The last-named described an example of a mixed C. histolytica and Histoplasma capsulatum systemic infection.

Recently Hassin [11] emphasized the rare and but thrice formerly encountered intracranial tumor composed of cryptococcic granulomas. His article includes an additional instance of cryptococcic meningoencephalitis, together with a report of a cerebellar tumor due to this organism. The latter form of tumefaction granuloma was likened to the lesions previously described by Dickenson, Veppo and Negri,[12] Swanson and Smith [13] and Smith and Crawford.[14] A still further example of this type of lesion might be found in a case reported by Wile [15] in 1935, of a 30 year old man with epileptiform seizures of four

4. Freeman, W.: Jahrb. f. Psychiat. u. Neurol. **43**:236, 1931. Freeman, W., and Weidman: Arch. Neurol. & Psychiat. **9**:589, 1923.

5. Levin, E. A.: Arch. Int. Med.: **59**:667, 1937.

6. Binford, C. H.: Am. J. Clin. Path. **11**:242, 1941.

7. Voyles, G. Q., and Beck, E. M.: Arch. Int. Med. **77**:504, 1946.

8. Mezey, C. M., and Fowler, R.: J. A. M. A. **132**:632, 1946.

9. Kraimer, L.; Small, J. M.; Hewlitt, A. B., and Deness, T.: J. Neurol., Neurosurg. & Psychiat. **9**:158, 1946.

10. Mider, G. B.; Smith, F. D., and Bray, W. E.: Arch. Path. **43**:102, 1947.

11. Hassin, G. B.: J. Neuropath. & Exper. Neurol. **6**:44, 1947.

12. Dickenson, G.; Veppo, A., and Negri, T.: Rev. neurol de Buenos Aires **7**:147, 1942.

13. Swanson, H. S., and Smith, W. A.: Arch. Neurol. & Psychiat. **51**:426, 1944.

14. Smith, F., and Crawford, J.: J. Path. & Bact. **33**:29, 1930.

15. Wile, U. J.: Arch. Dermat. & Syph. **31**:58. 1935.

years' duration, whose death was due to meningoencephalitic cryptococcosis. At autopsy the lesions in the meninges and the cerebrum showed varying degrees of chronicity and healing. In addition, there was a cicatrized lesion in the parieto-occipital cortex which consisted of a fibrotic mass containing cryptococci. This was considered the focus for the epileptiform seizures, which antedated death by more than four years and so predicates a minimal age to this once probably solitary lesion. The unusual persistence and longevity of the cryptococci in such focal scars had been observed by Weidman [16] in experimentally produced cutaneous cicatrices in monkeys.

Goodhart and Davison,[17] in their article of 1937, and Levin,[8] in the same year, indicated that all proved cases of cryptococcosis of the central nervous system of man had terminated fatally. However, Voyles and Beck [7] noted a disparity in the duration of illness in a series of cases and observed that in the few patients who survived the initial acute six month phase life was prolonged for two to five years. The factor of acquired immunity was suggested by these authors as being possibly present in the latter few cases. Hoff [18] attempted to produce immunity in mice by injecting killed cryptococci and succeeded in increasing the survival time of the mice but failed to ward off death by this procedure. One possible exception to the universal fatality of previously reported cases of cryptococcosis of the central nervous system, including also those instances of localized encephalitic granuloma referred to in Hassin's article, is to be found in the case reported by Mezey and Fowler.[9] At the time of the report, November 1946, the patient, was asymptomatic, showed no signs of meningeal involvement and had a negative result from the examination of the spinal fluid. However, none of these features, nor the entire combination, is unique in the sometimes protracted, intermittent course of the disease.

REPORT OF CASES

The present series of 3 cases represents solitary encephalitic cysts which contain cryptococcus-like organisms. These lesions were incidental findings in persons who died from other causes, and in each instance the crytococcic lesion presented the morphologic features of an arrested focal process. The patients' histories included no illness or cutaneous lesions usually attributed to this form of mycotic infection, and the terminal physical examinations gave no suggestive evidence

16. Weidman, F., in discussion on Wile.[14]

17. Goodhart, S. D., and Davison, C.: Arch. Neurol. & Psychiat. **37**:435, 1937.

18. Hoff, C. L.: J. Lab. & Clin. Med.: **27**:751, 1942.

of the presence of the cerebral lesions. The discussion of this unique form of infection of the brain will be prefixed by a report of 2 cases demonstrating the usual clinical and pathologic features seen in the meningoencephalitic process caused by this mycotic organism.

Case 1 (previously reported by Wade and Stevenson[19]).—A 39 year old German-American housewife sought hospitalization after three months of almost constant severe frontal headache, which had an insidious onset as fulness or pressure in the supraorbital regions. Lancinating nuchal pain had appeared during the several weeks prior to admission and was associated with paresthesia of the hands and back, weakness of the fingers and a heaviness about the axillas and breasts. On admission, physical examination disclosed an alert, robust woman in no distress. Neurologic examination revealed only slight weakness of the left side of the face and bilateral papilledema. An eczematoid cutaneous lesion present over the occiput yielded Staphylococcus aureus on culture. Roentgenologic examination of the chest and the skull and air ventriculograms brought out nothing that was significant. The results of routine studies of the peripheral blood were within normal limits. The spinal fluid, specimens of which were taken repeatedly, contained a number of cells ranging from 30 to 125, with a high predominance of lymphocytic forms. Cryptococci were not recognized. The spinal fluid protein level was 100 mg. per hundred cubic centimeters; the chloride level, 640 mg.; sugar was present in an undeterminable small quantity. The Wassermann reaction was positive on one occasion but negative on two subsequent examinations, and the colloidal gold curve was flat.

Two weeks were consumed in ridding the occipital region of the eczematoid lesion. During this period, no localizing signs or symptoms appeared, but the headache became more intense as papilledema progressed. These were but temporarily relieved by temporal decompression and letting of ventricular fluid. The diagnosis of mycotic meningitis was first entertained at craniotomy, on visualization of the ashen gray, opaque leptomeninges over the edematous cerebellar hemispheres. This impression was confirmed when C. histolytica was demonstrated in the meninges of a biopsy specimen of the cerebellum and later by cultural technic. Death occurred early in the postoperative period.

On gross neuropathologic examination the brain had a fairly thick, whitish gray exudate in the leptomeninges over the pons, cerebellum, interpeduncular fossa, temporal poles and frontal lobes. The exudate was present in a lesser degree over the thoracic and lumbar segments of the spinal cord but was not grossly present about the cauda equina. The convolutions of the cerebral hemispheres were moderately flattened, and there was a slight internal hydrocephalus.

On microscopic examination the meningeal exudate consisted principally of mononuclear round cells (many of them lymphocytes), some phagocytes and plasma cells. Nissl stain demonstrated many yeast organisms, both free in the leptomeninges and enclosed in large multinucleated cells. The organisms varied in size, some being twice as large as others, and the largest having diameters of approximately 20 microns. They were round and often surrounded by a clear zone. Sections through the temporal lobe showed many small cystic areas near the surface in the gray matter (fig. 1). These contained phagocytes and yeast organisms, together with a few small mononuclear cells resembling lymphocytes. The silver carbonate preparation for microglia cells demonstrated a distinct increase in the number of these elements throughout the gray matter of the cortex, even

19. Wade, L. J., and Stevenson, L. D.: Yale J. Biol. & Med. **13**:467, 1941.

where no lesions were visible, and the microglia cells were enlarged in many cases. Sudan III preparation showed small spherical globules of fat of different sizes within the yeast organisms. These appeared to be confined to the central sphere of the organism and were not within the clear halo. There were also collections of compound granule ("gitter") cells, filled with fat, about many of the blood vessels, in both the white and the gray matter. Many of the ganglion cells contained amounts of lipochrome pigment in marked excess of normal for the age of the patient.

The general autopsy disclosed a small (1.5 cm.) cyst in the lower lobe of the left lung. This had a dense fibrotic wall and yielded no cryptococci on culture and contained none histologically.

Fig. 1 (case 1).—Section through the temporal lobe showing a small encephalitic cyst and a thick meningeal exudate (× 15).

Case 2.—The protracted illness of the patient, a 36 year old white man, first became manifest in general malaise and fatigue approximately two and one-half years prior to death. Early in his illness he experienced short episodes of severe retro-orbital pain and anorexia and had mild persistent pyrexia (temperature, between 99 and 100 F.). A sudden attack of generalized headache, mental confusion, profuse hidrosis, nausea and vomiting brought him to the hospital about two years before his death. At that time examination revealed slight haziness of the optic disks, slight nuchal rigidity and urinary retention. Laboratory studies included roentgen examination of the chest, which appeared normal, urinalysis, serologic tests for Brucella infection and typhoid, and blood culture. The spinal fluid was under slightly increased pressure. It contained 144 cells, with a differential count of 94 per cent lymphocytes; also 151 mg. of protein, 52 mg. of sugar

and 726 mg. of chloride per hundred cubic centimeters. The colloidal gold curve was flat, and the serologic test for syphilis was negative. No growth was obtained on aerobic and anaerobic cultures. The blood, as tested serologically at the National Institute of Health, yielded negative reactions against encephalitic viruses. Air encephalograms demonstrated a mild degree of dilatation of the lateral ventricle.

After six weeks of hospitalization and a month of convalescence at home, the patient returned to his job as a pharmacist, directing a research program for a pharmaceutical house. Fatigue and infrequent, short, transitory lapses of memory were the sole complaints during the subsequent two years.

One month before entering the New York Hospital, the patient contracted an infection of the upper respiratory tract with a cough productive of yellowish

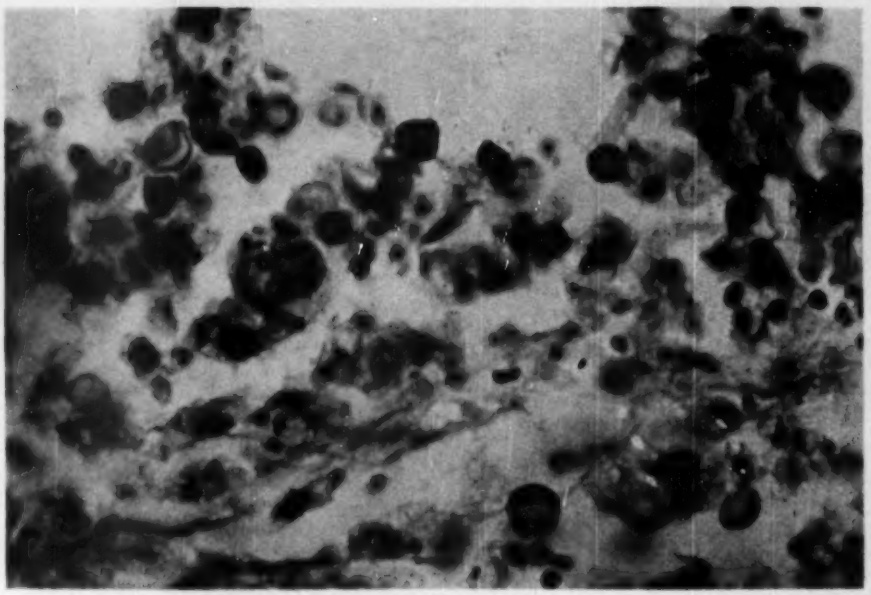

Fig. 2 (case 2).—An exudate in the leptomeninges containing numerous C. histolytica organisms, many within multinucleated giant cells (× 699).

gray sputum. There were blockage of the nasal passages and severe headache. Difficulty in motor control of the legs and disturbed equilibrium were noted during the next week. Mental confusion and urinary incontinence ensued on the day prior to hospitalization. On admission, physical examination revealed an asthenic white man who showed mental confusion but was oriented and did not appear particularly ill. There was neither nuchal rigidity nor papilledema, but mild photophobia. The gait was generally steady but was interrupted infrequently by episodes of staggering toward the right. A test for Romberg's sign was negative, and the remainder of the neurologic examination, the general physical examination, the roentgen examination of chest and skull, the urinalysis and the routine studies of the blood gave negative results. The clear spinal fluid was under a pressure of

Fig. 3.—Arteritis in a medium-sized pial vessel showing obliteration of the lumen by granulomatous tissue made up of lymphocytes, epithelioid cells and multinucleated giant cells (× 200).

120 mm. of water. It contained 199 cells, of which 97 per cent were lymphocytic forms. The protein varied from 129 to 165 mg. per hundred cubic centimeters, while the sugar and chloride levels were within normal limits. The colloidal gold curve was flat, and the Wasserman test was negative. A ventriculogram again disclosed mild dilatation of the lateral ventricles, behind nonfilling third and fourth ventricles.

The patient's mental status deteriorated rapidly during the next two weeks, without the development of localizing neurologic signs or symptoms. Hourly periods of generalized muscular rigidity and myoclonia appeared. Under these dire circumstances an exploratory craniotomy was performed. There was obstruction of the foramens of Monro, and this was considered to be due to ependymitis. On the subsequent day, concomitant with death, a culture of a previously taken specimen of spinal fluid yielded a growth of C. histolytica.

At autopsy, the brain grossly showed some thickening of the arachnoid over the pons and interpeduncular space and to a lesser degree over the hemispheres and the spinal cord. There were numerous small white tubercle-like masses in the arachnoid, principally over the interpeduncular space, but also along the basilar artery and over the lower part of the pons and the spinal cord. The convolutions of both hemispheres were moderately flattened, and there was a moderate degree of internal hydrocephalus. The cerebral veins were congested, and there were small areas of subarachnoid hemorrhage. An operative defect was present in the middle portion of the right frontal lobe. There was subependymal thickening, principally in the third ventricle, but it extended to involve the lateral ventricles as well.

Microscopically, the exudate observed in the leptomeninges was made up of lymphocytes, an odd neutrophilic granulocyte, many large giant cells and large epithelioid cells, with numerous cryptococci scattered throughout and contained within many of the giant cells (fig. 2). This exudate was more abundant about the brain stem and less obvious over the hemisphere and the spinal cord. In addition, there was distinct arteritis, principally of the medium-sized vessels of the pia mater. In these the intima was greatly thickened with fibrous tissue and the lumens were almost occluded by a cellular exudate, which in places formed granulomatous masses containing many epithelioid cells and giant cells as well as great numbers of cryptococci (fig. 3.). There was a minimal amount of encephalitis, with a few lymphocytes in the perivascular spaces about some of the cerebral vessels. No calcification or cyst formation was noted.

A solitary small fibrotic lesion was present in the lower lobe of the left lung, and numerous small nodules were strewn generously throughout the liver and the spleen. These presented the morphologic features of healed granulomas, although they did not yield cryptococci on culture and contained none histologically.

CASE 3.—A 72 year old Italian-born man was admitted to the hospital with the signs and symptoms of acute myocardial infarction. The history of past illnesses was rather incomplete but included no episode which could be construed as suggestive of a systemic or a cerebral mycotic infection.

The postmortem examination disclosed, as the cause of death, severe atherosclerosis of the coronary arteries with thrombotic occlusion and recent myocardial infarction. There were similarly severe atherosclerosis of the cerebral vessels and multiple small cerebral anemic infarcts.

The neuropathologic examination further showed a 1 cm. hard cystic nodule situated within the right putamen, lying tangential to the external capsule (fig. 4). Histologically, the lesion was composed of a thick fibrous tissue capsule and contained a mass of granular material, within which were numerous spheroid bodies (fig. 5). These had the appearance of cryptococci and varied in diameter

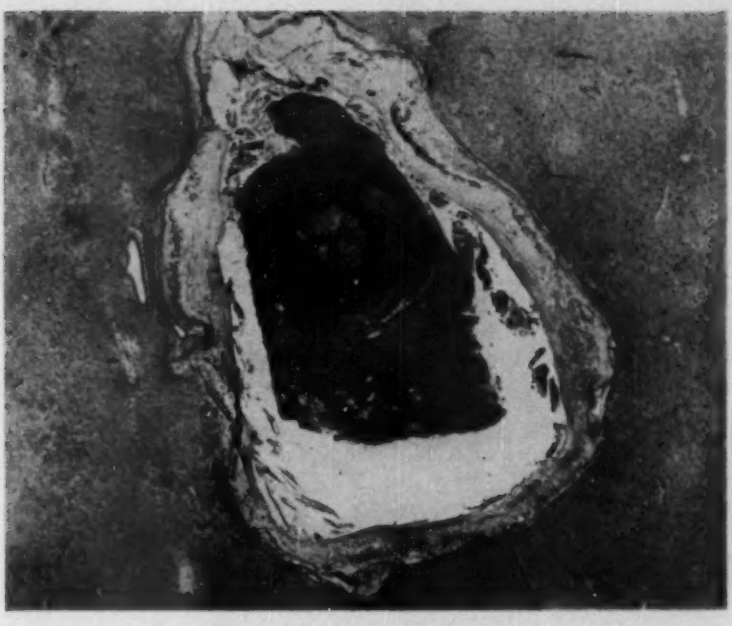

Fig. 4 (case 3).—Section through the right putamen showing a solitary cyst composed of an outer fibrous capsule and containing granular material (× 20).

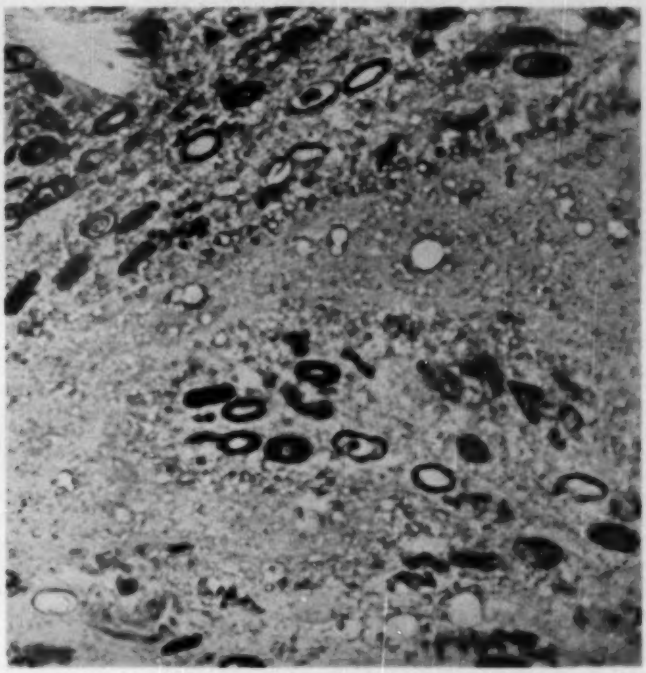

Fig. 5 (case 3).—Numerous C. histolytica organisms within the granular contents of the cyst (× 625).

from 10 to 20 microns. Cholesterol crystals were also contained within the cyst. There was a mild neuroglia reaction by the astrocytes of the brain surrounding the lesion.

CASE 4.—The patient was a 91 year old Hungarian-born man, who sought medical attention because of a rapidly enlarging mass in the upper part of the abdomen, associated with loss of weight and gastrointestinal symptoms. There was no history of illness referable to the central nervous system.

At autopsy, a large carcinomatous growth was found to involve extensively most of the abdominal organs and was considered to have arisen probably in the gallbladder.

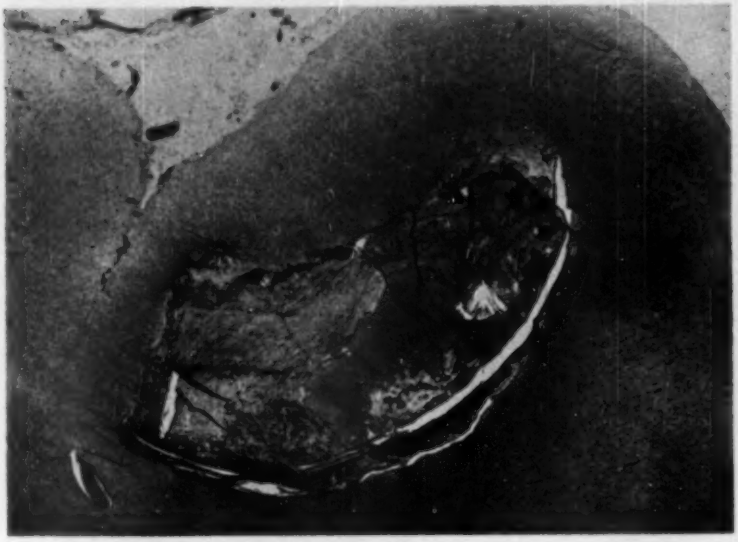

Fig. 6 (case 4).—Cyst within the gray matter of the right paracentral gyrus (× 20).

Neuropathologic examination disclosed a firm round nodule, 6 mm. in diameter, in the right hemisphere, involving the upper portion of the right paracentral lobule. Histologic sections (fig. 6) showed it to be an irregularly shaped oval cyst with a thick collagenous tissue capsule. In the outer layers of the fibrous mass there was a narrow cleft lined by small oval bodies varying in diameter between 8 and 25 microns. Although many appeared as silhouette-like forms, others had the morphologic aspects of C. histolytica. The fibrous tissue also contained cholesterol crystals, small blood vessels and focal collections of lymphocyte and plasma cells.

CASE 5.—A 55 year old Italian-born man had been hospitalized on several occasions for cardiac decompensation, with the clinical diagnosis of arteriosclerotic heart disease. His history included no noteworthy neurologic symptoms or illnesses suggesting involvement of the central nervous system.

The postmortem examination yielded, as the cause of death, severe atherosclerosis of the coronary arteries with a recent myocardial infarction involving the interventricular septum.

In the neuropathology laboratory, a solitary 1 cm. spheroid lesion was encountered in the cerebral hemisphere in the medial portion of the tip of the left frontal lobe (fig. 7). This had a soft granular interior and was surrounded by a firm gray capsule. Microscopically, the cyst contained cellular debris and cholesterol crystals. Its capsule was formed of dense collagenous tissue, within which were several multinucleated giant cells. Many spheroid bodies were harbored within the cyst,

Fig. 7 (case 5).—Cyst within the cortex of the left frontal lobe (× 20).

and a few were contained within its capsule. These, for the most part, retained no protoplasmic or organoid structures other than an outer shell or membrane. Nevertheless, they were morphologically quite similar, both in size and structure, to the less well preserved organisms seen in the lesions in the previous 2 cases.

COMMENT

When the central nervous system is invaded by the yeast C. histolytica, there results a variety of neurologic manifestations, which are diagnostically characteristic only in their bizarreness and their usually

protracted and often intermittent course. The pathologic process is similarly variable, but most commonly it takes the form of meningoencephalitis, in which a very characteristic feature is the formation of small encephalitic cysts, as exemplified in the first case. A second frequent finding is represented in the second case, which showed severe arteritis characterized by the formation of tubercles within and about the pial blood vessels.

The power of the central nervous system to cope with this organism has been viewed with extreme pessimism, although arrested lesions of a similar granulomatous nature are not infrequently seen in the solitary tuberculoma, while scars of healed cryptococcic infections have many times been described in the lungs and elsewhere. The solitary cysts cited in the latter 3 cases were incidental findings on postmortem examination and were apparently equally incidental and inconsequential clinical infections. Identification of the etiologic organism was necessarily based totally on morphologic aspects, although these are considered characteristic enough to be diagnostic with reasonable accuracy. The three lesions showed many common features and were indeed similar in size. All were formed of a thick, sparsely cellular capsule which contained a few lymphocytes, an occasional multinucleated giant cell and always cholesterol crystals. Granular débris, detritus and cholesterol crystals were the constant contents of these cysts. The organisms were located both within the contents of the cyst and in its capsule; they had excited little glial response. In none of the cases were there noteworthy alterations of the leptomeninges or stigmas denoting previous meningeal involvement.

SUMMARY

Solitary encephalitic cysts were found incidentally in the brains of 3 human beings who died of unrelated causes. Histologic studies showed that the cysts contained Cryptococcus histolytica. In each instance there was a thick fibrous capsule about the cyst, indicating that the process was arrested. The lesions provide examples of self-limited and clinically unsuspected cryptococcic encephalitis. Their existence widens the common concept that cryptococcosis of the central nervous system is a universally fatal meningoencephalitic process.

NEEDLE BIOPSY OF THE LIVER

V. Observations on the Distribution of Alkaline Phosphatase and Its Diagnostic Significance

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SINCE 1939, when Gomori [1] published his method of histochemical demonstration of alkaline phosphatase, there have been a number of reports concerning the distribution of this enzyme. In spite of several studies of this nature made with tissues from normal and diseased livers, the diagnostic significance of the varying amounts of phosphatase and of the manner in which it is distributed in the liver in sundry diseases still remains undetermined. The present paper concerns itself with an attempt to elucidate this problem.

MATERIAL AND METHOD

A study of 132 consecutive, unselected biopsy specimens of livers with respect to the distribution of alkaline phosphatase was undertaken in the gastrology laboratory of the Cincinnati General Hospital. Each specimen was obtained by the needle technic previously described.[2] The tissue thus obtained was divided, and one half of it was fixed in formaldehyde solution U. S. P. (formalin) sectioned in paraffin and stained in the usual manner with hematoxylin and eosin. In a modification of the Gomori technic, the second half of the biopsy specimen was fixed in cold acetone, sectioned in paraffin and incubated in a buffered sodium glycerophosphate solution for two hours at 40 C. This treatment of tissue in which there is active phosphatase allows the liberation of PO_4 ions and precipitation of calcium phosphate, which is insoluble. The precipitate occurs at the site of enzyme activity. The addition of cobaltous nitrate yields cobalt phosphate, which may be made visible by the reaction of ammonium sulfide, which results in the production of fine black cobalt sulfide granules at the sites of phosphatase activity.

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1. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23, 1939.

2. Kumpe, C. W.; Gall, E. A.; Schiff, L.; Molle, W. E.; Safdi, S. A., and Steinberg, H. H.: Gastroenterology **9**:672, 1947.

The sections stained with hematoxylin and eosin were utilized for diagnostic purposes and for anatomic orientation. The acetone-fixed fragments were studied with a view to establishing the distribution of alkaline phosphatase in the various disorders. One hundred and twelve specimens were deemed suitable for comparative analysis. The others were discarded because either the histochemical reaction was imperfect or the biopsy specimen was inadequate. The 112 specimens were grouped in accordance with the various lesions encountered. Twenty-four specimens represented histologically normal livers. Twenty-two specimens showed diffuse hepatitis, 26 portal cirrhosis, 14 fatty liver, 10 obstructive bile stasis and 7 neoplastic disease; in the remaining 9 there was a miscellany of lesions.

HISTOLOGIC OBSERVATIONS

Normal Livers (24 specimens).—The subjects from whom the normal specimens were obtained suffered from heart disease, chronic pulmonary disease, arthritis or intestinal disorders. The serum bilirubin in these patients was less than 1.0 mg. per hundred cubic centimeters in all cases and the serum alkaline phosphatase was less than 5 Bodansky units per hundred cubic centimeters in all but 2 of the instances in which it was determined. The 2 exceptions had serum alkaline phosphatase readings of 7.2 and 7.8 units, respectively.

Liver cells showed phosphatase activity primarily within nuclei, where it was indicated by the deposit of fine black granules on nuclear membrane, nucleolus and chromatin. The phenomenon was only infrequently observed in the cytoplasm. There was no apparent lobular localization of the enzyme.

The intercellular bile canaliculi exhibited focal activity in every specimen, with only a narrow range of variance in the amounts (fig. 1).

The sinusoids revealed varying degrees of the enzyme in their walls, the precipitate being most prominent in the region adjacent to the central vein and both less abundant and more variable in the middle and peripheral zones. The endothelium of the central veins was uniformly active.

Within the portal triads, constant enzymatic activity was noted in the endothelium of the portal vein and the hepatic artery. In bile duct epithelium, only the nuclei showed activity, and this was slight and variable. The mature connective tissue was inactive. The nuclei of the lymphocytes within the portal areas were uniformly active, a feature also evident in polymorphonuclear leukocytes, but not in Kupffer cells (fig. 2).

Diffuse Hepatitis (22 specimens).—Twenty of the patients in this group had infectious (viral) hepatitis, 1 patient had homologous serum hepatitis and 1 had arsenical hepatitis. Histologically, 16 patients (these included the patients with serum and arsenical hepatitis) were found to have an active, florid lesion; in 5 the process was healing, and 1 patient with active infectious hepatitis also had early postnecrotic

cirrhosis. The lesions of serum and arsenical hepatitis differed in no perceptible manner from that of infectious hepatitis.

Intact liver cells were identical in phosphatase activity with those in the normal group, and degenerating and necrotic cells exhibited no increase in activity. The sinusoids were universally active and presented a more uniform appearance than those in the control series (fig. 3).

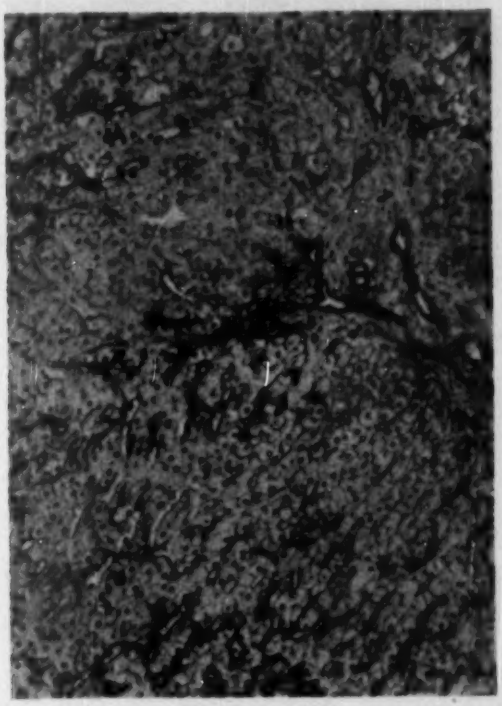

Fig. 1.—Normal liver: Phosphatase granules are present in the nuclei of the liver cells, in the endothelium of the central vein (*A*), in centrally and peripherally located sinusoids and in the endothelial elements of the portal triad (*B*). The fine black lines outlining the hepatic cells are the biliary capillaries containing the enzyme. (× 160.)

Evidence of intrasinusoidal phosphatase activity was accentuated in the vicinity of liver cell necrosis (fig. 4). This was of such a degree that cellular detail was obscured focally by the precipitated black granules. This was particularly striking in the more severe degrees of hepatitis with extensive cellular necrosis (fig. 5), and it was thought to be a

result of increased enzyme activity in the endothelium of the sinusoids, and not actually an increase within the deteriorating liver cells.

The intercellular bile canaliculi differed in no way from those in the normal tissues. The ductules occasionally were active, as were inspissated bile plugs when present. In general, however, the precipitated bile stained yellow or greenish brown, with only a few black granules

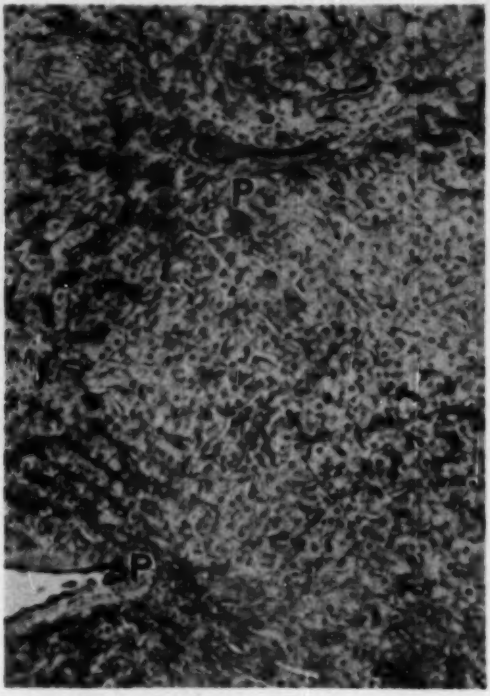

Fig. 2.—Normal liver: Alkaline phosphatase with normal distribution but showing focal increase in the periportal sinusoids (*P*). The cytoplasm of the liver cells exhibits no enzymatic activity. (× 160.)

appearing. Bile pigment within liver cells gave no evidence of phosphatase content.

There was an accentuation in the degree of activity in the portal triads, which was attributed to closer approximation of sinusoids following necrosis of adjacent liver cells and collapse of the supporting reticulum. The endothelium of the portal vein and the hepatic artery and the epithelium of the bile ducts reacted as in the normal state.

Leukocytes and proliferating fibroblasts showed activity, the enzyme being wholly nuclear in location.

The essential differences, then, in infectious hepatitis as compared with the normal liver were the increased local content of phosphatase within the sinusoids adjacent to degenerating and necrotic liver cells and the wider distribution of the enzyme in the walls of the sinusoids.

Fig. 3.—Healing hepatitis: Black granules evidencing phosphatase activity appear in the walls of all sinusoids without the zonal variation seen in normal liver. Biliary capillaries are present as pale fine lines demarcating the liver cells. (× 160.)

Portal Cirrhosis (26 specimens).—The findings in 26 cases of cirrhosis were subclassified pathologically as nutritional in 13, postnecrotic in 9, pigmentary in 1 and unclassified cirrhosis in 3. Of these 26 cases, the lesion appeared to be active and progressive in 25 and inactive in 1.

As elsewhere, phosphatase was present in the nuclei of the liver cells but not in the cytoplasm. This was also the case in cells distended with fat vacuoles. Degenerating and necrotic cells showed no increase of staining but were often obscured by the dense black granulations of the adjacent sinusoids. There was patchy phosphatase activity in the biliary canaliculi.

Fig. 4.—Acute diffuse hepatitis (viral): Great increase of alkaline phosphatase is exhibited in the portal area and in the periportal sinusoids. The liver cells show a normal content. In the lower left portion of the illustration the cellular detail is obscured by the local increase of the stromal enzyme (arrow). (× 160.)

The sinusoids showed a slight to striking increase in enzyme activity, this being most evident at the periphery of the lobules, where there were dense black deposits. The lining of the central veins exhibited a variable increase of phosphatase, and in the perilobular portal areas proliferating fibroblasts showed nuclear and, on occasion, even some cytoplasmic enzyme (fig. 6). Nuclei of the epithelium of the biliary ductules were

unchanged, but endothelium lining the portal veins showed increased activity.

In general, areas of active hepatic cirrhosis were the seat of increased phosphatase activity, which reached its greatest intensity in the post-necrotic type (fig. 7). In the progressive disease the apparent increase of enzyme content was due only indirectly to degeneration and necrosis

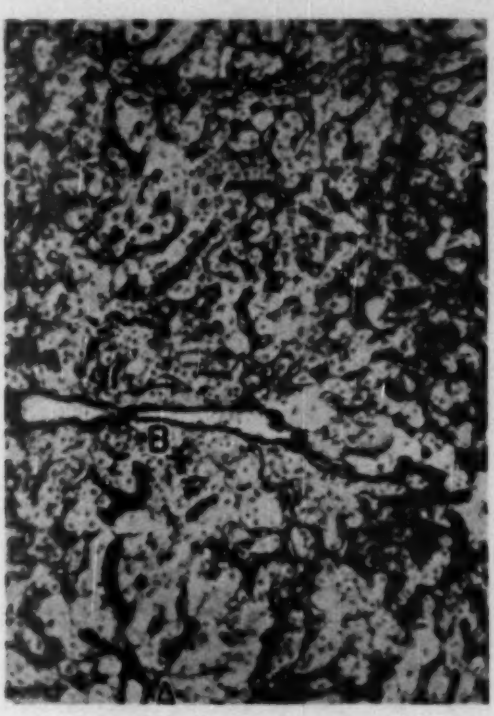

Fig. 5.—Acute diffuse hepatitis (viral): The diffuse increase of the enzyme is shown in the sinusoids, the central vein (*A*) and the portal area (*B*). (× 160.)

of liver cells. Actually there was a larger number of sinusoids per unit area as a result of the compression and destruction of liver cells by contracting fibrous tissue. In liver showing inactive cirrhosis the distribution of phosphatase was essentially the same as in normal liver.

Obstructive Bile Stasis (10 specimens).—Among the 10 patients in this group there was calculus of the common bile duct in 2, carcinoma of the head of the pancreas in 2, metastatic carcinoma in 1 and hepatic

duct carcinoma in 1. The cause of the obstruction was not established in 4 cases. The serum bilirubin content varied from 10 to 53 mg. per hundred cubic centimeters and the serum alkaline phosphatase from 7.5 to 39.5 Bodansky units.

As in all other instances, in this group liver cells showed phosphatase activity only in the nuclei, and the biliary canaliculi revealed patchy

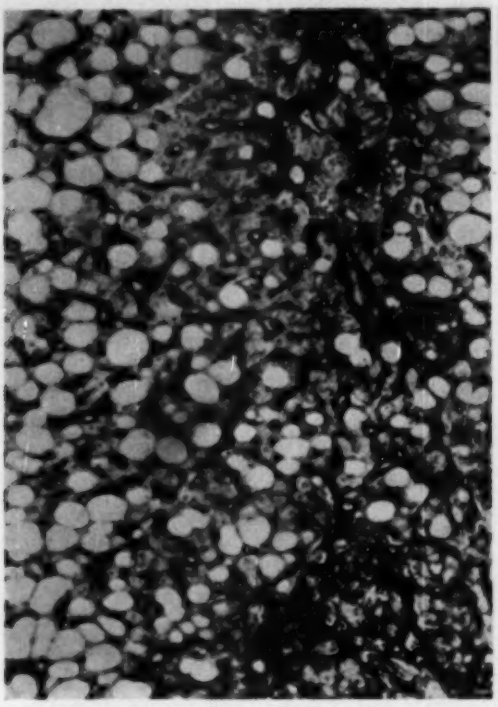

Fig. 6.—Nutritional cirrhosis: An increase of phosphatase is displayed in the periportal sinusoids. No increase is evident in either the normal or the fat-distended liver cells. Focally there is increased activity in the biliary canaliculi outlining the liver cells. (× 160.)

activity. Here, however, the canalicular activity was more extensive than in normal liver, being particularly striking in the vicinity of the most severe stasis of bile (fig. 8). Bile plugs exhibited phosphatase in the majority of instances but not in all. Intracytoplasmic bile pigment contained no enzyme.

The lining of the sinusoids stained diffusely, thus differing from the normal lining, in which there is zonal activity. The concentration was greatest in the regions of most marked stasis of bile and in the peripheral segments immediately adjacent to the portal areas. In fact, the activity seen in the periportal sinusoids was more intense than that in any region previously encountered. The central veins, however, showed

Fig. 7.—Postnecrotic cirrhosis: In the lower portion of the field there is a portal islet showing abundant fibrosis and increased phosphatase in the endothelium of the portal vein and the artery. Nuclear enzymatic activity only is seen in the fibrous tissue and in the epithelium of the bile ductules. The distorted groups of liver cells show the normal content of phosphatase, although the sinusoids exhibit a striking increase in enzyme granules. (× 160.)

only slight increase of enzyme, and in the portal triads the content was essentially the same as in the normal.

Neoplastic Disease (7 specimens).—There were 6 cases of carcinoma; in 5 it was metastatic, and in 1 there was a primary cholangioma. In addition, there was 1 case of cancerous lymphoma.

The distribution of phosphatase was essentially that of the normal liver with the exception that in the presence of biliary obstruction due to neoplasm, the changes were similar to those seen in the cases of obstruction. In the 6 cases of carcinoma there was no detectable enzyme activity in the neoplastic cells. In the case of cancerous lymphoma, which was of the lymphocytic type, the neoplastic cells did show nuclear

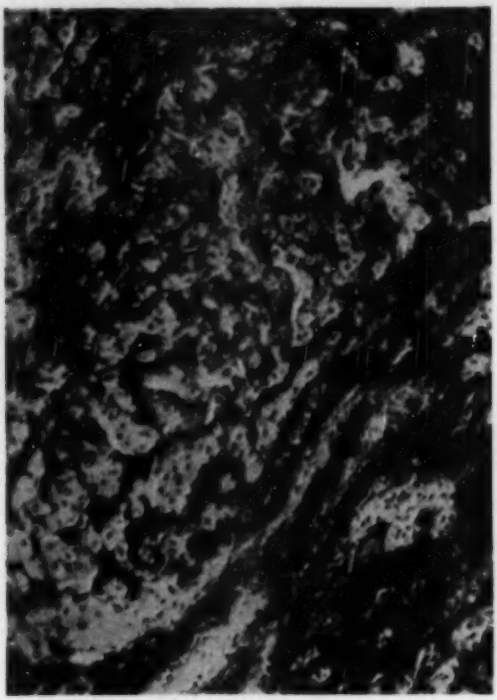

Fig. 8.—Obstructive bile stasis and bilary cirrhosis: Sinusoids throughout the area reveal intense phosphatase activity. Intercellular biliary canaliculi also show focal increase of the enzyme, and inspissated bile plugs are active. (× 200.)

phosphatase in amounts essentially similar to those seen in lymphocytes of inflammatory nature.

Fatty Vacuolation (14 specimens).—Twelve of the 14 patients in this group had a history of severe alchoholism and/or a deficient diet. The other 2 patients suffered from myocardial infarction. In all instances the parenchymatous elements contained fat to a moderate or a conspicuous degree.

The distribution of the black granules differed in no respect from that seen in the normal specimens. Occasionally there was an irregular increase in the endothelium of the sinusoids. The liver cells, distended with fat vacuoles, contained the enzyme in the displaced nuclei only and in essentially normal amounts.

Miscellaneous Group (9 specimens).—Clinically the diseases presented by the 9 patients in this group were considered to be diagnostic problems. The different possibilities entertained were tuberculosis, brucellosis, neoplasm, infectious hepatitis and infectious mononucleosis. The pathologic observations were of varied character, the lesions being

Distribution of Phosphatase and Amounts, Revealed by Degrees of Activity, in Normal and in Diseased Livers

	Normal Liver (34)	Diffuse Hepatitis (22)	Portal Cirrhosis (36)	Fatty Vacuolization (14)	Obstructive Bile Stasis (10)	Carcinoma (6)	Lymphoma (1)	Miscellaneous (9)
Liver cell:								
Nucleus	+	+	+	+	+	+	+	+
Cytoplasm	+	+	+	+	+	+	+	+
Sinusoids	+	++	+++	++	+++	+	+	+
Central vein	+	++	++	+	+++	+	+	+
Bile canaliculi	+	++	++	+	+++	+	+	+
Portal areas:								
Portal vein	+	+	++	+	+++	+	+	+
Hepatic artery	+	+	++	+	+++	+	+	+
Bile duct								
Nuclei epithelium	+	+	+	+	+	+	+	+
Miscellaneous:								
Fibrous tissue								
Young	+	+	+	+	+	+	+	+
Adult	0	0	0	0	0	0	0	0
Lymphocytes	+	+	+	+	+	+	+	+
Kupffer cells	0	0	0	0	0	0	0	0
Polymorphonuclear leukocytes	+	+	+	+	+	+	+	+

Active = +; inactive = 0; increased = ++ - +++.

termed chronic pericholangitis in 4 instances, miliary granuloma in 4 and nonspecific focal hepatitis in 1.

In pericholangitis the distribution of alkaline phosphatase was similar to the normal. In the portal areas only the inflammatory cells showed nuclear activity, which was slight. This was true also in the case of miliary granulomas. In the case of focal hepatitis the necrotic liver cells showed no increase of enzyme activity.

COMMENT

The purpose of this study has been twofold. Primarily it has been our intent to determine whether or not the phosphatase is distributed in the diseased liver in a pattern sufficiently characteristic to be of value

in morphologic differential diagnosis. The incidental purpose has been to extend the observations of other investigators in this field.

The table demonstrates what we have found to be the average distribution of alkaline phosphatase in the normal liver and in seven categories of diseased livers. The trend of phosphatase activity within the several groups is readily apparent; however, the table does not reflect the wide variations from case to case within each of the groups.

In the normal liver samples there was a rather wide range of phosphatase activity with considerable variation from case to case. This has been recognized and described by previous workers. Gomori [3] stated that the capillary endothelium is more or less constantly positive, and this has been observed in the present series. Although Sherlock [4] had noted a rather uniform appearance of the enzyme in both nuclei and cytoplasm of the hepatic parenchyma, we found little evidence that the enzyme was present in the cytoplasm in our material. Our observation of the variable amounts of phosphatase in biliary capillaries is in agreement with those of Gomori [3] and of Wachstein and Zak.[5] Bourne [6] stated in his report concerning the distribution of alkaline phosphatase that the biliary capillaries of human liver did not exhibit enzymatic activity.

In healing hepatitis the distribution of the enzyme often simulated the normal, differing only in the increased extent of activity in the sinusoids, particularly in the vicinity of degeneration and necrosis of liver cells. Sherlock observed an increased amount of enzyme in necrotic cells, whereas Wachstein and Zak [7] saw no increase in necrotic cells of specimens obtained post mortem. Takamatsu,[8] who in 1939 also developed a method for the histologic demonstration of alkaline phosphatase similar to that of Gomori, described increased activity in damaged liver cells but gave no details. Wachstein,[9] observing experimental animals which had been subjected to cytoplasmic toxins, recorded no increase of alkaline phosphatase occurring within the degenerating and necrotic liver cells. Our own experiences have paralleled those of Wachstein.

Bile stasis due to obstruction resulted in an increase of phosphatase in the areas of stagnation, and here the activity was naturally, therefore, more intense than in normal livers. Most of the bile plugs contained

3. Gomori, G.: J. Cell. & Comp. Physiol. **17**:71, 1941.
4. Sherlock, S. P. V., and Walshe, V.: J. Path. Bact. **59**:615, 1947.
5. Wachstein, M., and Zak, F. G.: Arch. Path. **42**:501, 1946.
6. Bourne, G.: Quart. J. Exper. Physiol. **32**:1, 1943.
7. Wachstein, M., and Zak, F. G.: Proc. Soc. Exper. Biol. & Med. **62**:73, 1946.
8. Takamatsu, H.: Tr. Soc. path. jap. **29**:492, 1939.
9. Wachstein, M.: Arch. Path. **40**:57, 1945.

phosphatase, although intracytoplasmic bile granules showed no enzyme. Sherlock also found the enzyme in the bile thrombi, but Wachstein and Zak [7] did not observe its occurrence in their material. The sinusoidal phosphatase was more abundant in lesions involving the periportal areas than in any other hepatic lesion. Wachstein and Zak [7] produced biliary obstruction in dogs and were able to demonstrate dilatation of biliary capillaries and an increase of enzyme granules about them.

The variations we have observed in portal cirrhosis resulted only from the retrograde changes in the hepatic parenchyma or from the contraction associated with the fibrosis. Fatty vacuolation and focal lesions of the liver had no influence on the over-all pattern of phosphatase distribution. The only changes occurring in neoplastic involvement of the liver were those resulting from obstruction of bile flow and compression of the liver cells. In the single case of cancerous lymphoma, phosphatase activity was observed in the nuclei of the tumor cells. Kabat and Furth [10] found no phosphatase in the neoplastic cells in a case of acute lymphoid leukemia.

It would appear from our experiences that the patterns of alkaline phosphatase distribution shown in the biopsy specimens of livers could serve no great diagnostic purpose. One of the basic morphologic problems in the interpretation of the biopsy specimen of liver is the distinction between obstructive and hepatogenous jaundice. In the majority of cases the histologic structure noted in routine sections is sufficiently distinctive to permit clearcut differentiation. In the occasional case in which the lesion cannot be classified unequivocally, the phosphatase pattern appears to be of too indefinite and variable a nature to be of diagnostic aid. It is felt, however, that the continued histochemical study of the degrees to which this enzyme occurs in the liver may elucidate the problem of its morphogenesis.

SUMMARY AND CONCLUSIONS

An attempt is made to determine the value of the alkaline phosphatase activity of the liver in the morphologic differential diagnosis of specimens obtained by the needle biopsy technic. Unfortunately, the relatively wide variation from case to case served only to indicate disease trends in groups of cases rather than in individual instances.

Fatty vacuolation of liver cells and focal lesions of the liver did not alter the over-all distribution of phosphatase.

The phosphatase content was increased in infectious hepatitis and was similarly increased in the active phases of portal cirrhosis. In the inactive stage of the latter there was normal distribution.

10. Kabat, E. A., and Furth, J.: Am. J. Path. **17**:303, 1941.

Obstructive bile stasis produced the most intense increase in phosphatase of any hepatic disease.

The enzyme was not present in carcinoma cells but was present in the nuclei of neoplastic lymphocytes in essentially the same amounts as seen in normal lymphocytes.

A study of the manner in which alkaline phosphatase is distributed in the biopsy specimen of liver adds little of diagnostic significance. The procedure may prove fruitful, however, in further investigation designed to reveal the source of alkaline phosphatase and the functional status of the hepatic parenchyma in disease states.

Case Reports

CARDIOESOPHAGEAL ADENOACANTHOMA

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CONSIDERABLE attention has been directed in recent years to a group of epithelial neoplasms showing a peculiar combination of pavemental and glandular histologic patterns. Sporadic cases in which this type of neoplasm appeared in different parts of the body have been reported in the literature under various names.

The tumor has been known as malpighian epithelioma,[1] adenocancroid,[2] adenosquamous carcinoma,[3] polymorphous epithelioma [4] and adenoacanthoma. The last term apparently has been generally accepted in the American literature,[5] and since it describes the tumor as well as the others, we shall continue using it.

In 5 of the 60 cases of adenoacanthoma reported by Nochimowski [6] the lesion was in the stomach wall; in none of the 60 cases was the esophagus involved. Strassman [5c] reviewed all the cases of gastric adenoacanthoma. He listed 12 cases and added 1 of his own. In only 2 of these [7] did the adenoacanthoma involve the entire stomach, including the cardia. In most of the others it was confined to the pylorus (9 cases) and to the body of the stomach (2 cases). McPeak and Arons [8] reported in 1947 a case in which adenoacanthoma was confined to the esophagus. The tumor reported by these authors presented certain characteristics of salivary gland carcinoma, and an attempt was made to correlate its

From the Laboratory Service and Surgical Service, Veterans Administration Hospital, Richmond, Va.

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1. Gautheir-Villars, P., and Leger, L.: Ann. d'anat. path. **16**:1065, 1940.

2. Herxheimer, G.: Beitr. z. path. Anat. u. z. allg. Path. **41**:348, 1907.

3. Rabson, S. M.: Arch. Path. **21**:308, 1936.

4. Oberling, C., and Wolf, M.: Bull. Assoc. franç. l'étude du cancer **16**:68, 1927.

5. (*a*) Pasternack, J. G.: Am. J. Path. **11**:541, 1935. (*b*) Wood, D. A.: Arch. Path. **36**:177, 1943. (*c*) Strassman, G.: ibid. **41**:213, 1946. (*d*) Moore, R. A.: Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1945, p. 204. (*e*) McPeak, E., and Warren, S.: Am. J. Path. **24**:971, 1948.

6. Nochimowski, J.: Ztschr. f. Path. **44**:547, 1933.

7. Takagi, C.: Gann **31**:173, 1937. Strassman.[5c]

8. McPeak, E., and Arons, W. L.: Arch. Path. **44**:385, 1947.

histogenesis with that of neoplasms arising from salivary glands. The same authors refer to cases in which adenoacanthoma involved the esophagus [9]; however, no detailed description or illustrations pertaining to these cases were available.

Several observers [10] stated that adenoacanthoma occurring in the cardioesophageal junction was not unusual. A similar impression was gained by us on discussing the issue with several pathologists. However, we could find in the literature reports of only 2 cases in which the tumor was observed at this location, and possibly arose there.[11]

McPeak and Warren [5c] reported a series of cases of tumor of the cardioesophageal junction and reviewed the morphologic features of carcinoma arising in this location. Eight of their cases were tentatively classified as instances of adenoacanthoma. Four of the specimens were obtained at necropsies and 4 were removed surgically.

The morphologic features of the present tumor differed from those described by McPeak and Warren, and it was felt that a different histogenetic process had taken place in this instance.

Notkin [12] advocated that cardioesophageal neoplasms be grouped apart from gastric and esophageal tumors in order that accurate studies of the evidence concerning them might be facilitated. The statistical reports of gastric and esophageal carcinoma are often inaccurate because the tumors occurring in the cardia are listed in either group.

REPORT OF A CASE

A 57 year old white man was admitted to the Veterans Administration Hospital, Richmond, Va., on Jan. 20, 1947. For five months prior to admission he had been troubled by dysphagia and vomiting, which were becoming progressively more severe. At the time of admission he was vomiting practically all the solid food ingested. He had lost 20 pounds (9 Kg.) during this illness. There had been no hematemesis or melena. His previous health had been good. He had not had any other serious illnesses, and his family history was noncontributory.

Physical examination revealed limitation of the expansion of his thoracic cage, and on auscultation the expiratory phase of his breath sounds was prolonged. Bilateral inguinal adenopathy was present, the glands being discrete and shotty. The other physical findings, such as bilateral pterygia and a moderate deformity of the proximal third of his right tibia and fibula resulting from an old simple fracture, had no bearing on the disease in question.

Barium sulfate studies of the esophagus made shortly after admission showed a marked irregular narrowing of the lower end with moderate dilatation above the narrowing. The stomach and duodenum appeared normal except for a 40 per cent gastric retention on the six hour film, which was believed to be due to the decrease in the inflow of the barium sulfate meal from the esophagus.

An esophagoscopic examination was made on January 29, and a nodular mass was visualized 36 cm. from the incisor teeth. There was a pencil-sized lumen

9. Cabot Case 21521, New England J. Med. **213**:1311, 1935; Cabot Case 19122, ibid. **208**:652, 1933.

10. Rahson.[9] Pasternack.[5a] Strassman.[5c]

11. Strassman.[5c] Takagi.[7]

12. Notkin, L. J.: Canad. M. A. J. **19**:96, 1928.

through the center of the lesion. The lesion did not appear to be fixed. The mucosa was smooth and shiny, and no ulceration was seen. Two small biopsy specimens were removed.

A bronchoscopic examination made immediately after this examination disclosed no lesions. There was no detectable fixation of the left stem bronchus.

Microscopic examination of the biopsy material revealed a strip of stratified squamous epithelium supported by connective tissue and strands of smooth muscle. This was heavily invaded by disarranged glandular tissue composed of tubular and acinous elements. The glandular epithelium varied from cuboidal to columnar, with a moderate amount of cytoplasm. The nuclei of the cells differed considerably in size, shape and staining intensity. There was considerable evidence of mitotic

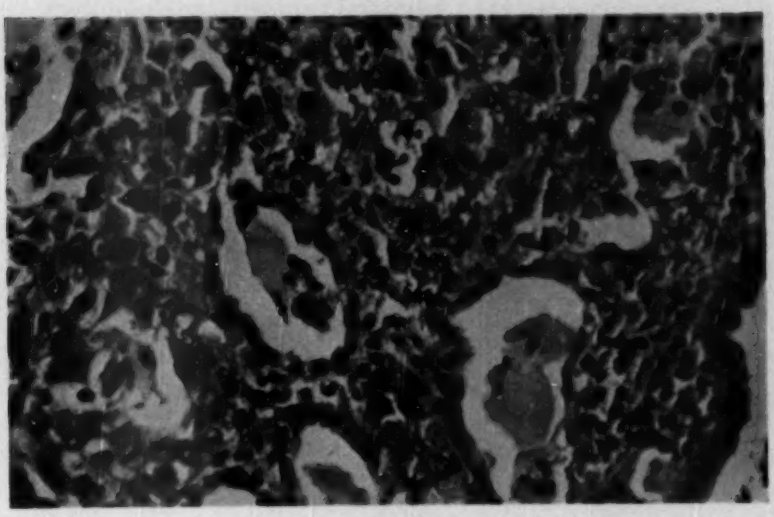

Fig. 1.—Neoplastic glandular elements; × 200.

activity; some of the mitotic figures were atypical. A diagnosis of esophageal adenocarcinoma was made.

On February 6 thoracotomy was performed. A bulky lesion was encountered which involved the lower end of the esophagus and a portion of the cardia of the stomach. Many large firm lymph nodes were present in the gastrohepatic ligament and about the celiac axis. A few somewhat smaller hard nodes were present along the greater curvature. There was extensive involvement of the periesophageal tissue. The lower end of the esophagus and the cardia of the stomach were resected, and esophagogastrostomy was performed. The spleen was removed with the cardia. At the time of operation it was felt that this procedure would be of only palliative value because of the extensive involvement of the adjacent lymph nodes and periesophageal connective tissue.

Description of the Tumor.—The resected segment of the esophageal tube was distorted by a firm nodular tumor that involved the entire circumference of the organ and from 6 to 8 cm. of its length. The tumor replaced most of the mucosal layer. It encroached on the lumen of the organ, reducing it to a tortuous slit at some points; at others the lumen remained patent, with an eroded lining and rigid walls. The muscular layer was greatly hypertrophied, the circular layer measuring up to 6 mm. in thickness. The proximal end of the tumor faded imperceptibly into the normal mucosa, while the lower edge formed an elevated nodular ridge. The cardia could not be identified but a rosette-like collar of normal gastric wall formed the lower end of the specimen. The gross appearance did not furnish any clues to the peculiar histologic nature of the growth.

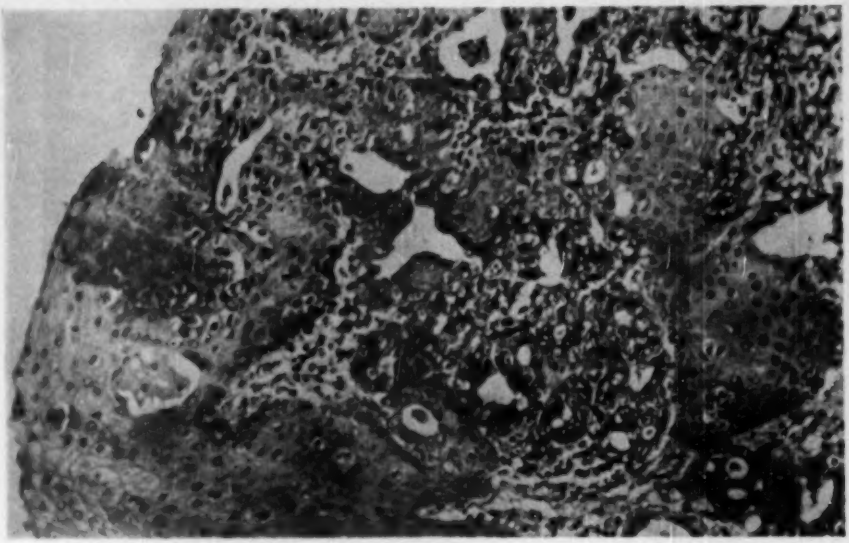

Fig. 2.—Mixture of glandular and squamous elements; × 120.

Numerous blocks from various levels and from various sectors of the viscus were sectioned.

The microscopic examination revealed a mixture of two types of growth. The prevailing part of the tumor in the proximal segment of the specimen was formed by a maze of disarranged tubular and acinous elements supported by a scanty, well vascularized stroma (fig. 1). The glandular lumens contained an eosinophilic homogeneous material, taken to be a secretory product. The glandular structures were lined by epithelium varying from low cuboidal to columnar, with considerable distortion of individual cells. The cytoplasm was homogeneous and eosinophilic. The nuclei differed in shape, size and density. Mitotic activity was pronounced, with atypical mitotic figures well in evidence. Scattered among the glandular

elements were islands of neoplastic squamous epithelium (fig. 2). In the proximal part of the specimen (fig. 3) these were scanty downgrowths arising from the remnants of the lining, while in the distal portion (fig. 4) the epidermoid elements prevailed. The epidermoid tissue was not merely a manifestation of pseudo-epitheliomatous hyperplasia. It showed definite characteristics of a cancerous growth, such as pleomorphism, atypical mitotic activity and bizarre nuclei. In a

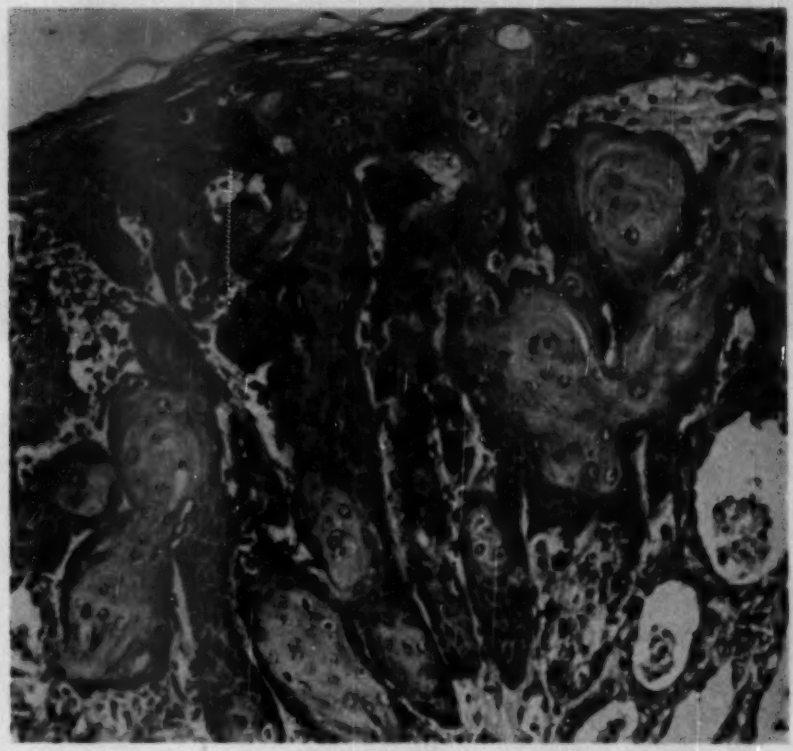

Fig. 3.—Epithelial downgrowths originating from the esophageal lining; × 120.

few areas the tumor presented a papillary appearance (fig. 5). Some of the papillary fronds were thin, with a delicate stalk, while others were supported by a dense fibrous stroma. The cells lining the papillary projections were mostly columnar, with hyperchromic basophilic cytoplasm and considerable anisonucleosis. Some of the papillary structures projected into microcystic spaces formed apparently by anastomosis of similar papillae. The passing of the squamous epithelium into stratified columnar and simple columnar epithelium could be observed in these areas. The lining pavemental epithelium seemed to creep down

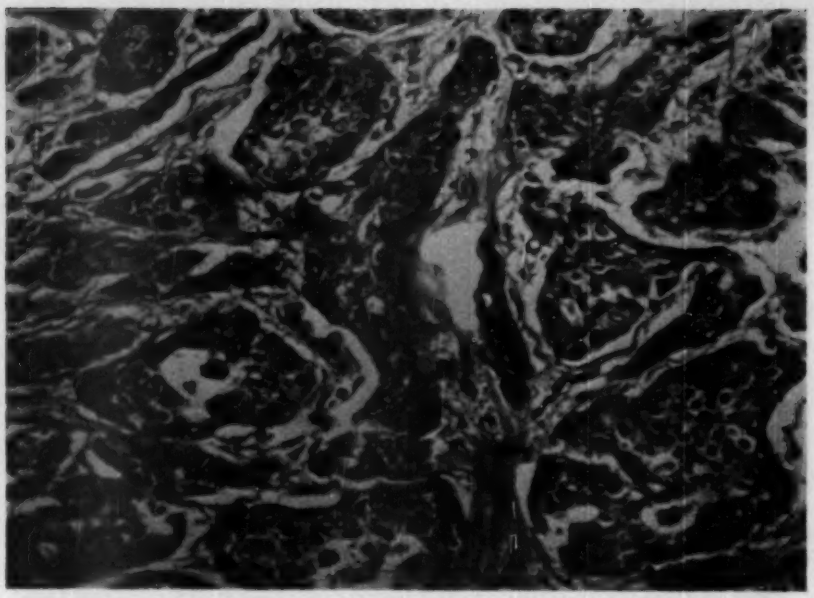

Fig. 4.—Part of tumor composed exclusively of epidermoid neoplastic elements; × 120.

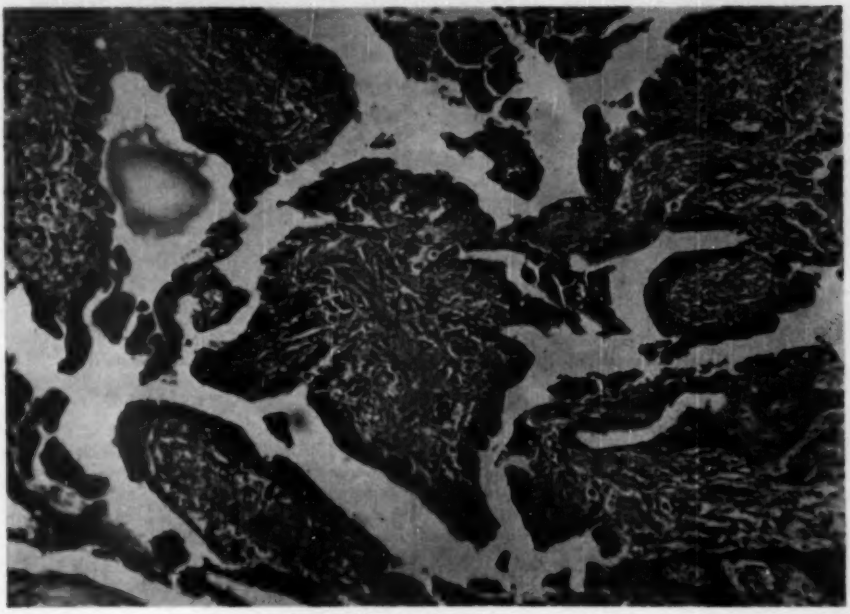

Fig. 5.—Papillomatous features of the tumor; × 100.

along the shores of narrow invaginations to merge with the columnar epithelium covering the papillary projections and lining the microcystic spaces. A hypertrophic muscular coat permeated by strands and nests of tumor tissue was seen in some of the sections. In view of the peculiar mixture of epidermoid and glandular carcinomatous elements the lesion was diagnosed as gastroesophageal adenoacanthoma.

Postoperative Course.—The patient's postoperative course was uneventful. He was discharged from the hospital on March 13, thirty-five days after the operation. During his convalescence he underwent two surgical procedures (transplantations of bilateral pterygia). He was completely asymptomatic and able to take a regular diet at this time. He was instructed to report to a Veterans Administration Hospital in the event that he had further difficulties. The patient's further history was obtained through the courtesy of the staff of Baker Veterans Administration Hospital, Martinsburg, W. Va., where he was admitted on Aug. 7, 1947, six months after his operation. He stated that he got along well for three months postoperatively and continued to gain weight for three months, until May 1947, when he noted pain in the right paravertebral area of the chest. The pain became progressively worse. About two weeks prior to his last admission the patient noted a small nodule appearing under his skin. He did not seem acutely ill. There was a firm nodule about 5 cm. in diameter on the right paravertebral area at the level of the fifth rib. A biopsy of this nodule revealed a fragment of skeletal muscle invaded by epidermoid carcinoma. The glandular and papillary elements of the primary growth did not reproduce themselves. Only in a few of the nests did central degeneration occur, these nests then simulating ductlike structures. The nodule was tender and fixed. Cutaneous sensation over this area was reduced. Firm subdermal nodules were felt in the abdominal wall superior to the umbilicus and in the right lower quadrant of the abdomen. There were also hard nodules, about 2 cm. in diameter, in the right biceps and one attached to the proximal end of the right humerus. Roentgenograms of the skeletal system revealed marked hypertrophic arthritis but no evidence of skeletal metastases. A roentgenogram of the stomach revealed a distortion of pattern; however, it was impossible to determine whether this was the result of the surgical procedure or was due to a recurrence of the neoplasm. The skin over the lower extremities was somewhat edematous. The patient grew progressively weaker and on the thirty-fifth day of his last hospitalization he became short of breath and cyanotic. He rapidly passed into coma and died within a few hours after the onset of his respiratory difficulties. Permission to perform necropsy was not obtained.

COMMENT

Many hypotheses have been evolved in an attempt to explain the development of adenoacanthoma.

Lubarsch [13] mentioned the possibility of true metaplasia of normal mucosal cells preceding the development of neoplastic potentialities or occurring simultaneously.

13. Lubarsch, O.: Verhandl. d. deutsch. path. Gesellsch. **10**:198, 1906.

Herxheimer [2] expressed the belief that the cancerous squamous and adenomatous cells develop from undifferentiated embryonic cell groups rather than from foci of more mature ectopic cells.

Oberling and Wolf [4] expressed the belief that in one of their 2 cases the neoplasm originated as a metaplasia of adult cells and that in the other it represented congenital heterotopy.

Stewart and Lorenz [14] were able to produce adenoacanthoma in the forestomach and in the pyloric stomach of mice with injections of methylcholanthrene. They concluded that a normal glandular mucosa could, under carcinogenic influences, redifferentiate into glandular and squamous cell neoplastic elements. This conclusion is warranted because squamous epithelium has never been demonstrated in the pyloric region of the stomach.

Wood [5b] concluded, after reviewing experimental studies, that adenoacanthoma develops as a result of direct carcinogenic stimulation. Once neoplasia is initiated it may, according to his belief, develop varying degrees of redifferentiation and develop not only columnar but also squamous cells.

Strassman [5c] advanced two possible theories to explain the presence of squamous epithelium in his case. According to him, it could represent congenital heterotopy of esophageal epithelium or, more likely, the squamous cells might have been derived from undifferentiated basal cells in the gastric mucosa which had undergone metaplastic changes.

Lubarsch [15] maintained that adenocarcinoma of the esophagus need not originate from ectopic gastric glands. He quoted instances in which benign ectopic gastric glands may be seen interspersed between areas of adenocarcinoma arising from mucinous esophageal glands, and he places the burden of proof on those who claim that esophageal adenocarcinoma originates from heterotopic gastric glands.

The adenoacanthomas reported by McPeak and Warren [5a] and by McPeak and Arons [8] were taken to be tumors of salivary gland type arising from esophageal glands.

In attempting to determine the histogenesis of the tumor in the present case it is impossible to rule out any of the hypotheses advanced by other authors.

The tumor patterns in our case could have arisen as a result of squamous metaplasia in neoplastic glandular tissue or as a result of dual differentiation of embryonic cell rests, some of the cells differentiating into glandular and others into pavemental neoplastic elements.

The histologic pattern of mixed tumors of the salivary gland type was not duplicated in our case.

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14. Stewart, M. L., and Lorenz, E. J.: J. Nat. Cancer Inst. **2**:193, 1941; **3**:175, 1942.

15. Henke, E., and Lubarsch, O.: Handbuch der speziellen Pathologie und Histologie, Berlin, Julius Springer, 1926, vol. 4, pt. 1, p. 144. Lubarsch.[13]

The location of the lesion in an area where squamous and diversified glandular epithelial elements are normally available suggests the probability of a polycentric origin of the tumor. Unknown carcinogenic factors influenced both types of epithelium at the gastroesophageal junction and stimulated the rise of two adjoining cancers. In the course of their growth and expansion these neoplasms collided, became closely interwoven and thus produced a mixed pattern. The fact that only one of the tumor patterns appeared in the metastasis corroborates this impression.

SUMMARY

A case of adenoacanthoma of the gastroesophageal junction is presented. The histogenetic theories are discussed and the possibility that adenoacanthoma may represent a polycentric growth or a collision of tumors is suggested.

DOUBLE VOLVULUS

A. PETER CRANE, M.D.
NORWALK, CONN.

BECAUSE I could find no description of simultaneous double volvulus of the cecum and the sigmoid in the available literature, I am reporting a case of this anomaly.

REPORT OF A CASE

The patient was an 84 year old white woman, first seen at another hospital in May 1945. Two months before she was admitted to that hospital, she suffered a fall, striking her abdomen. In consequence she had mild pain in the lower part of the abdomen, gradually becoming worse and accompanied by distention. One week before she entered the hospital, the pain localized in the left lower quadrant of the abdomen and became more severe. The abdomen became progressively more distended, and relief was not obtained with enemas or cathartics. For over fifty years the patient had taken cathartics regularly. There had been no loss of weight, no nausea or vomiting, no jaundice and no bloody or tarry stools. She had not undergone any abdominal operation. The temperature was 100 F.; the pulse rate, 100 per minute; the respiratory rate, 22 per minute. The blood pressure was 190 systolic and 94 diastolic. The patient was a thin, acutely ill, senile woman with tremendous abdominal distention, tympany and diffuse tenderness. No abdominal masses were felt. Urinalysis revealed no abnormality. A flat film of the abdomen was interpreted as showing intestinal obstruction, probably due to a localized cancerous lesion and volvulus at the junction of the descending and sigmoid portions of the colon. Three days after admission a Miller-Abbott tube was passed and fecal drainage obtained. A second roentgen examination six days later revealed collapse of the intestine proximal to the splenic flexure, but there was still a moderate amount of gas in the distal colon. The next day the abdomen was explored through an incision of the left rectus muscle. A simple 10 cm. cyst of the ovary was removed, and a volvulus of the sigmoid flexure was found. The operative note stated that the sigmoid and descending portions of the colon were particularly redundant and that the sigmoid portion was twisted so as to form a partial volvulus, which was not tight and unwound as the colon was straightened out. No other lesions of the bowel were found. Recovery was uneventful, and the patient was discharged eleven days after the operation.

In January 1947 she was seen at the Norwalk Hospital, under the care of Dr. Reuben I. H. Solway, where she entered because of an attack of mild epigastric pain and distention that was unrelieved by enemas. There had been no vomiting. The temperature was 99.8 F., the pulse rate, 100 per minute; the respiratory rate, 28 per minute. The blood pressure was 194 systolic and 100 diastolic. The abdomen was diffusely tender, tympanitic and extremely distended. The urine was normal except for a trace of albumin. The patient was given several enemas, without good results. Four days after admission a Miller-Abbott tube was passed and a large

From the Laboratory of Norwalk Hospital, Norwalk, Conn., and the Department of Pathology of Yale University School of Medicine, New Haven, Conn.

amount of fecal drainage obtained. The patient did poorly for a few days, but with continuous suction and administration of enemas and parenteral fluid, the distention subsided, and the patient was discharged ten days after admission.

She was admitted in September 1948 with the same complaints and physical findings. The urine contained albumin (1 plus), sugar (1 plus) and five white blood cells per high-powered field. The hemoglobin content was 13 Gm. (84.4 per cent); the white cell count, 5,000 with a differential count of 1 eosinophilic granulocyte, 4 band cells, 65 neutrophilic glanulocytes, 24 lymphocytes and 6 monocytes per hundred cells. She was treated as before, with relief of symptoms, and was discharged fourteen days after admission.

The last admission was four months later, in January 1949. At this time, in addition to generalized distention, she complained of pain in the right lower quadrant of the abdomen. Two days after admission she began having tarry stools, and this condition of the stools persisted for a few days. She was so feeble that

Double volvulus: *A*, position of the dilated loops of bowel on opening the abdomen; *B*, separation of the two volvuli, showing the upper loop (volvulus of the cecum) and the lower loop (volvulus of the sigmoid). The small intestines are crowded into the pelvis.

operation was not attempted. The course was downhill, and seven days after admission she died.

Necropsy (four hours after death).—The body was that of a thin, undernourished white woman, 63 inches (160 cm.) in height and weighing an estimated 100 pounds (45.5 Kg.). The abdomen was enormously distended, the skin over it being shiny and bluish white. In the left lower quadrant was a well healed scar of the region of the rectus muscle, 15 cm. in length. When the abdomen was opened, one encountered two enormous loops of bowel with thinned, purple-colored walls (*A* in figure). On separating these two loops (*B* in figure) one found that the lower was formed by a volvulus of the sigmoid which had rotated 360 degrees counterclockwise about the terminal fixed portion of the descending colon and the rectosigmoid. When the volvulus was untwisted, the sigmoid was an elongated redundant segment of bowel with a corresponding increase in mesentery. The

upper loop of bowel was a cecal volvulus. Using the ileocecal valve as a stationary point, the redundant and freely movable cecum had rotated counterclockwise 180 degrees. This caused the ascending colon to kink on itself where it was firmly attached above the ileocecal valve. The appendix was 4 cm. in length and was bound to the anterior surface of the cecum by fibrous adhesions. There was a small amount of black fecal matter in the sigmoid and descending colon. No free fluid was seen in the abdominal cavity. The small intestine had been pushed into the pelvis. There was no distention of the large or the small bowel except in the two volvuli. In microscopic sections taken from the involved bowel wall there was loss of the mucosa, thinning of the wall and great dilatation of the veins. There was, however, no cellular reaction in the bowel wall. In the pulmonary artery of the lower lobe of the right lung there was a well formed adherent thrombus which did not cause complete occlusion of the artery. The liver revealed focal central zones of necrosis. Other conditions noted were focal lipid pneumonia, generalized arteriosclerosis and scars of the kidneys.

COMMENT

Volvulus of the sigmoid is an unusual cause of acute intestinal obstruction in this country, the incidence varying from 1 to 4.5 per cent in several series.[1] Cecal volvulus is an even more unusual cause of acute intestinal obstruction, the incidence varying from 0.67 to 4.3 per cent.[2] This incidence is decidedly less than that in Eastern Europe, Russia and Scandinavia, where the incidence of volvulus as a cause of acute intestinal obstruction has been reported as greater than 50 per cent.[1b]

McIver [3] stated that a mobile loop of the sigmoid flexure is the basic factor in the production of a volvulus. Some authors [4] have found fibrous bands and adhesions in the mesentery, while others [5] have found nothing. Bloodgood [4a] expressed the belief that chronic constipation caused thickening of the bowel wall with elongation. The proximal and distal points of the sigmoid thus remained close together supporting the redundant loop of sigmoid, which could easily twist on itself. The marked distention of the twisted loop usually found has been attributed to anaerobic colonic bacteria.[6]

Volvulus of the cecum likewise depends on excessive mobility of that section of the bowel. Wolfer, Beaton and Anson [7] expressed the opinion that in addition to hypermobility there must also be a point of hyperfixation of the ascending colon. They found that 11.2 per cent of cadavers had this type of anatomic arrangement. The high residue

1. (*a*) Hays, G. L.: Ann. Surg. **19**:724, 1922. (*b*) Griffin, W. D.; Bartron, G. R., and Meyer, K. A.: Surg., Gynec. & Obst. **81**:287, 1945. (*c*) Metheny D., and Nichols, H. E.: ibid. **76**:239, 1943.

2. Sweet, R. H.: New England J. Med. **213**:287, 1935. Browne, D. C., and McHardy, G.: Am. J. Digest. Dis. **9**:177, 1942. Rothman, M.; Bruckner, J. P., and Zetena, D. F.: Am. J. Surg. **60**:292, 1943. Donhauser, J. L., and Atwell, S.: Arch. Surg. **58**:129, 1949.

3. McIver, M. A.: Am. J. Surg. **19**:378, 1933.

4. (*a*) Bloodgood, J. C.: Ann. Surg. **49**:161, 1909. (*b*) Hays.[1a]

5. Essenson, L., and Ginzberg, L.: Am. J. Surg. **77**:240, 1949.

6. Weinstein, M.: Ann. Surg. **107**:248, 1938.

7. Wolfer, J. A.; Beaton, L. E., and Anson, B. J.: Surg., Gynec. & Obst. **74**:882, 1942.

diets and periods of fasting and feasting of peoples in Eastern Europe are perhaps of significance in producing redundant segments of bowel in the relatively dependent cecum and sigmoid. Apparently, volvulus of the cecum is more commonly related to congenital, and volvulus of the sigmoid to acquired, anatomic defects. The excitant and immediate cause of the acute volvulus is not known but is probably related to diet and bowel habits.

From the history, our patient's volvulus clinically corresponds to the subacute type of volvulus of the sigmoid seen in the older age groups.[1b] Since there was no cellular reaction in either bowel wall, there is no way of definitely determining which of these acute volvuli occurred first. In view of the fact that the volvulus of the sigmoid was known four years before death, it is likely that the subsequent attacks were due to this condition. The pain occurring in the right lower quadrant of the abdomen as a new symptom in the final episode of her disease suggests that the cecal volvulus occurred first at that time. It is possible that the chronic obstruction due to the volvulus of the sigmoid helped produce redundancy of the cecum, and that in the final episode the pressure of the enlarged sigmoid caused rotation of the cecum.

SUMMARY

A case of double volvulus involving the cecum and the sigmoid flexure of the colon is reported.

News and Notes

News from Germany.—The following five news items were submitted by Dr. Howard Karsner on his return from Germany, where he served as overseas consultant for the United States Army.

R. Rössle has retired as professor of pathology at the University of Berlin and is now pathologist to the Tempelhof Krankenhaus, where he has a fine laboratory and has with him two of his former assistants and a secretary who has been with him for twenty-five years. He is editor of *Virchows Archiv für pathologische Anatomie und Physiologie und für klinische Medizin.*

R. Schmincke has retired as professor of pathology at the University of Heidelberg, where he has been succeeded by E. Randerath, formerly of Düsseldorf. Schmincke is of great aid to the Fourth Medical Laboratory of the United States Army.

A. Lauche is now professor of pathology at the University of Frankfurt, in the Senckenberg Institute, and is editing the *Frankfürter Zeitschrift für Pathologie.*

Werner Hueck has moved from Leipzig to become professor of Pathology at the University of Munich, succeeding Borst and occupying the partly destroyed institute built for Borst.

H. Chiari, professor of pathology at the University of Vienna, Austria, moved the specimens of the Rokitansky Museum to the basement in the early days of the war. The museum has now been beautifully refurbished in its old quarters, and the great collection is intact. In addition to the congenital heart diseases, there is the original specimen described by Rokitansky in 1852 as "Diathesis aneurysmatica" which has been shown to be polyarteritis nodosa of the mesenteric vessels.

New Research Laboratory at Brookhaven National Laboratory.—Brookhaven National Laboratory has opened two new research laboratories in the Medical Department, one for the Division of Bacteriology and Virology and the other for the Division of Pathology. The former division is headed by William M. Hale, formerly head of the department of bacteriology at the University of Iowa College of Medicine, and the latter division is headed by S. C. Madden, chairman of the department of pathology, Emory University School of Medicine, Atlanta, Ga.

Dr. Evans Receives Mickle Award.—For his researches in endocrinology, Dr. Herbert M. Evans, director of the University of California Institute of Experimental Biology, San Francisco, has received the Mickle Award of the Faculty of Medicine of the University of Toronto. The award is made once every ten years "to that individual who has contributed most significantly and in a practical way to the advance of medicine in any of its fields." Dr. Evans received the award for "a long-continued series of studies relating to the isolation and final purification of five of the six anterior hypophyseal hormones."

Board Examinations.—The spring examination of the American Board of Pathology will be held in Madison, Wis., April 11 and 12, 1950. Applications for this examination will be received until March 15, 1950.

The fall examination will be held in St. Louis, Nov. 10 and 11, 1950.

Appointments.—Carl L. Larson, assistant chief of the Laboratory of Infectious Diseases, National Institutes of Health, Bethesda, Md., has been appointed head of the Rocky Mountain Laboratory, Hamilton, Mont. Dr. Larson succeeds the late Ralph R. Parker.

John F. Sheehan, chairman of the department of pathology in the Stritch School of Medicine of Loyola University, Chicago, has been made acting dean of the school in the place of James M. Smith, who has resigned.

Abner Golden has been appointed assistant professor of pathology at Emory University School of Medicine, Atlanta, Ga., and pathologist at Emory University Hospital, effective Jan. 1, 1950.

Society News.—The following officers of the Oklahoma Association of Pathologists have been elected for the year 1949-1950: Leo Lowbeer, Tulsa, president; I. H. Nelson, Tulsa, vice president; Charles D. Tool, Edmond, secretary.

Deaths.—Preston Kyes died Dec. 27, 1949, in North Jay, Maine. Dr. Kyes was professor emeritus of preventive medicine at the University of Chicago School of Medicine. He was at one time assistant professor of experimental pathology at the same institution and was an editor of the University of Chicago's Science Series.

CORRECTIONS

In the notice of Dr. Bonnelle W. Rhamy's death in the December 1949 issue of the ARCHIVES OF PATHOLOGY, page 594, Dr. Rhamy was incorrectly called Dr. William R. Bonnelle.

In the first instalment of the article entitled "Choriocarcinoma" by Drs. W. Wallace Park and James C. Lees, in the January issue (ARCH. PATH. **49**:73, 1950), "table 4" should be "table 6" at the end of the second paragraph on page 92 and at the beginning of the second paragraph on page 95. In the second line on page 94 "page 55" should be "page 215."

Books Received

NORMAL VALUES IN CLINICAL MEDICINE. By F. William Sunderman, M.D., Ph.D., professor of experimental medicine and clinical pathology at the University of Texas Postgraduate School of Medicine and chief of the department of clinical pathology and director of clinical research at the M. D. Anderson Hospital for Cancer Research, Houston, Texas; and Frederick Boerner, V.M.D., late associate professor of clinical bacteriology at the University of Pennsylvania Graduate School of Medicine, and assistant professor of bacteriology at the University of Pennsylvania School of Medicine, now bacteriologist at the Graduate Hospital of Philadelphia and advanced bacteriologist in the Pennsylvania Department of Health. Pp. 845, with 236 figures and 413 tables. Price $14. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London W.C.2, 1949.

This volume goes a long way toward filling a real need in medicine, namely, a compendium of normal values. The authors have compiled an immense amount of material, which is well classified, ordered and arranged. The book is divided into sections, each dealing with some system, as follows: circulatory system, blood cellular elements, chemical properties of blood, respiratory system, digestive system, nervous system, urinary system, male and female genitalia, skeleton, skin and appendages, eye, ear, nose, endocrine, metabolism, anatomic norms, teeth and saliva, and miscellaneous. Most sections, in turn, are divided into separate chapters, each of which deals with a specific topic within the larger field. Acknowledgment is made to a large group of collaborators who offered help and guidance in the selection of appropriate material.

The book covers essentially the entire body, not so much organs as fields of knowledge, such as cardiology, neurology, gynecology and obstetrics, rhinology and the like. It is obvious that some fields are more susceptible to quantitative arrangement of data than are others. Clearly the material of the sections dealing with chemistry or chemical data can be concisely and succinctly treated. The chapters dealing with the blood, the urine, and the gastrointestinal system, matters which are so largely chemical in nature, together with the tabulations concerning metabolism and nutrition, anatomic values, and miscellaneous data, are highly successful. On the other hand, much of the material on the circulatory system, the nervous system and the special senses, is far less successfully managed. It may well be questioned whether anatomic figures, photomicrographs, roentgenograms, the elements of electroencephalography, or electrocardiography, an analysis of nerve reflexes and other bits of anatomic or physiologic knowledge belong in a book of this type. It is not possible, within the confines of a single volume, to convey a complete education. This book cannot substitute for a textbook of anatomy, physiology, physical diagnosis or endocrinology. Perhaps it has been too ambitious.

The book contains an amazing amount of data, excellently arranged, well classified and instantly available. To this reviewer it appears unfortunately to contain a considerable amount of material, of explanatory and descriptive character, which adds greatly to its expense and bulk, without adding correspondingly to its usefulness. All knowledge is of value, but perhaps, for the sake of reducing the rather high price, some of the material could have been omitted. The volume deserves a wide circulation.

HISTOPATHOLOGY OF THE SKIN. By Walter G. Lever, M.D., instructor in dermatology, Harvard Medical School; assistant dermatologist, Massachusetts General Hospital; associate in dermatology, Peter Bent Brigham Hospital; consulting dermatologist, Massachusetts Eye and Ear Infirmary. Pp. 449, with 221 illustrations. Price $10. J. B. Lippincott Company, 227-231 S. 6th, Philadelphia, 1949.

In this beautifully illustrated textbook of dermopathology the topics are appropriately arranged in the following sequence: embryology and histology of the skin; congenital diseases; noninfectious inflammatory diseases; eruptions due to drugs; degenerative diseases; bacterial, spirochetal, fungal, protozoal and viral lesions; metabolic diseases of the skin, including the so-called "collagen" diseases (one chapter), and, finally, epidermal and mesodermal neoplastic lesions, including the lymphomas and leukemias.

Under the heading of each disease process there is a brief clinical note, which is followed by an ample description of the microscopic aspects of the lesion and this in turn by a morphologic differential diagnosis. Titles of published articles pertinent to the subjects under discussion are listed at the ends of the chapters. They are largely drawn from the English medical literature. There is a complete subject index at the end of the book. The type is clear, and the illustrations exceedingly well chosen and amazingly free of defects.

Many pathologists will undoubtedly be inclined to disagree with the author regarding his classification of tumors of the skin and his views of their origin and pathogenesis, with reference particularly to basal cell carcinoma. This, however, need not detract from the intrinsic value of this textbook. The general pathologist will find it most useful as an aid in the difficult and sometimes bewildering task of diagnosing cutaneous lesions from prepared sections.

FORENSIC SCIENCE AND LABORATORY TECHNICS. By Ralph F. Turner, associate professor, Department of Police Administration, Michigan State College; formerly laboratory supervisor, Police Department, Kansas City, Mo. Price $6.50. Pp. 240, with 82 illustrations and 4 tables. Charles C Thomas, 220 E. Monroe St., Springfield, Ill., 1949.

Serologic and chemical methods of examining various materials, including human, have been known for quite some time. In forensic science the utilization of these technics whereby laboratory methods are coordinated with police work is comparatively recent. In fact, the combining of these various procedures in a scientific crime detection laboratory was the result of the St. Valentine day massacre which occurred in Chicago in 1929. Civic-minded persons then realized that a laboratory for scientific crime detection was an essential unit in the study of forensic medicine.

Dr. Turner has assembled in his book the important laboratory technics that are useful in the study of crime detection. In the first chapter, on photography, emphasis is placed on the equipment necessary to obtain good photographs, for these may play an important role as evidence. Since this book is primarily intended for police investigation, there is a chapter on the recording of a crime scene and another on the collecting and preserving of evidence. The chapter on fingerprints and firearms is also within the realm of police investigation. For the pathologist, the chapters on hair, fibers, chemistry, serologic tests and the examination of minute pieces of evidence are of interest. There is finally a chapter on documents.

This treatise undoubtedly serves as an important contribution to all who are interested in the laboratory technics of forensic science. If a forensic laboratory functions properly and the findings are accurate, reports of examinations may be the deciding factor in establishing the innocence or guilt of the person or persons

involved. The pathologist is not primarily a police or legal investigator. However, in the performance of autopsies, particularly medicolegal necropsies, the pathologist must concern himself with such findings of the forensic laboratory and the general police investigations as will aid him in his studies.

Dr. Turner's book is profusely illustrated, and the illustrations are clear. The subject matter is easy to read. Emphasis is placed on the necessity for speed plus accuracy in police science.

DISEASES OF THE HEART. By Charles K. Friedberg, M.D., associate physician, Mount Sinai Hospital, New York; lecturer in medicine, Columbia University. Pp. 1081, with 79 illustrations. Price $11.50. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London W.C.2, 1949.

This book is to be commended for the dynamic, physiologic approach to heart disease and its complications. The literature on each subject is reviewed well, and the bibliographies are extensive. With relatively little iteration, diseases and clinical phenomena are discussed under separate headings according to etiologic factors, pathologic aspects, clinical manifestations and physiologic pathogenesis. The descriptions and discussions of the large problems of coronary arterial, hypertensive and valvular disease, of the digitalis preparations and their applications, and of the management of congestive heart failure are all excellent. These chapters are thorough and should be stimulating to students. They are replete with sound clinical discussion and advice for practitioners, though many may feel that the value of oxygen has been underestimated. The chapters on circulatory measurements in congestive failure and on acute circulatory failure are comprehensive and valuable. Less common and rare diseases of the heart are treated briefly but well, and add to the completeness and interest of the text.

The weaknesses of this text stem from the attempt at thoroughness. The brief discussion of ventricular gradient and vectorcardiograms presupposes much knowledge of electrocardiography. The roentgenographic and electrocardiographic illustrations are well chosen, but often the textual descriptions of electrocardiographic changes are detailed and may be confusing to the uninitiated. The early chapters devoted to physiologic considerations contain obscurities and apparent contradictions of thought and nomenclature which may confuse the student and bewilder the practitioner who is not familiar with the literature. However, the wealth of information and discussion is not appreciably impaired by these defections from the general excellence of this first edition.

DAS ADENOCARCINOMA DES COLLUM UTERI: HISTOLOGISCHE, KLINISCHE UND THERAPEUTISCHE ERGEBNISSE (Adenocarcinoma of the Cervix Uteri with Histologic, Clinical and Therapeutic Details). By Hans Limburg and Klaus Thomsen, Gynecologic Division, University of Hamburg-Eppendorf. Pp. 87, with 38 illustrations. Georg Thieme, Diemershaldenstrasse 47; Stuttgart-O, Germany, 1949.

Adenocarcinoma forms only a small percentage of all carcinomas of the cervix, variously estimated from 2.7 to 13.5. This type of carcinoma is generally summarily dismissed in textbook accounts of neoplasms of the cervix. A monograph dealing exclusively with adenocarcinoma of the cervix is therefore welcome.

The authors were able to collect 65 examples over a twenty-eight year period, comprising 3 per cent of all cancers of the cervix. Histologically these were divided into the following six groups: (1) fully and moderately differentiated, (2) undifferentiated, (3) papillary, (4) Gärtner's duct type, (5) mucinous and (6) adenoacanthomatous. In contrast with the common epidermoid type of carcinoma of the

cervix, adenocarcinoma is from the outset infiltrative, and symptoms are late in appearing. For this reason, adenocarcinoma of the cervix has been associated with a poor prognosis.

A strong plea is made for elective therapy of adenocarcinoma of the cervix rather than any fixed procedure. Despite the view that this tumor is radioresistant, the authors have found that the judicious use of combined radium and roentgen ray therapy may yield a 21.4 per cent five year survival rate in cases in which, for the most part, the tumor was unsuitable for operation. In the cases in which it was operable, a 40.5 per cent five year cure rate was obtained. While the total percentage of five year cures for 53 cases of adenocarcinoma was 28.3 per cent and that of 1,202 cases of epidermoid carcinoma 29.8 per cent, the unfavorable character of the adenocarcinomatous lesion is indicated by the fact that the cure rates for the clinical groups 1 and 2 of adenocarcinoma were only 52.6 and 25 per cent, respectively, whereas for epidermoid carcinoma they were 77.7 and 56 per cent, respectively.

A Textbook of Neuropathology with Clinical, Anatomical and Technical Supplements. By Ben W. Lichtenstein, M.D., M.S., associate professor of neurology, University of Illinois College of Medicine; state neuropathologist, Illinois Neuropsychiatric Institute; attending neurologist, Cook County Hospital; professor of neurology, Cook County Graduate School of Medicine; attending neuropsychiatrist, Mount Sinai Hospital, Chicago. Pp. 474, with 282 illustrations. Price $9.50. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London W. C. 2, 1949.

This textbook is designed especially for the medical student and for those in training for the practice of neurology, psychiatry, pathology and neurologic surgery. The author has called on a large experience and mostly his own material in writing this treatise. Diseases are classified along anatomic and pathologic lines, so that entities are grouped under the headings of degeneration, inflammation, hemorrhage, hyperplasia, neoplasia and malformations. This approach fits the need of the pathologist but is more complex than an etiologic classification. The illustrations are numerous, original and of high quality. Every attempt has been made toward clarity of labeling and demonstration. Numerous up-to-date references, classified, are presented at the end of each chapter. There are three supplements to the text. The first of these presents syndromes, paralyses and uncommon diseases not grouped under other headings, for the convenience of the clinically minded. The second is a brief neuroanatomic summary. The third supplement gives an excellent and complete listing of neuropathologic technics.

Because of the method of classification of material and the scholarly approach, this book may prove somewhat difficult for the medical student beginning his study of diseases of the nervous system. However, it is an excellent guide and reference book for the pathologist, the neurologist and the psychiatrist.

Die Dystrophie (Dystrophy). By Prof. Dr. Med. Heinrich Berning, head of the department of medicine of the Universitätsklinik, Hamburg-Eppendorf, Germany (director, Prof. H. H. Berg). Cloth. Pp. 197, with 34 figures and 15 tables. Georg Thieme, Diemershaldenstrasse 47, Stuttgart-O, Germany; Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1949.

This monograph deals with malnutrition (dystrophy) observed in 200 patients during the period from 1943 to 1948. Under the term "dystrophy" the author includes those conditions associated with a profound disturbance of protein metabolism and previously known under different names, such as "epidemic dropsy," "nutritional edema" and *Hungerkrankheit* (hunger sickness).

After two introductory chapters presenting the historical aspects and the biochemical basis of this condition, the symptoms and the laboratory findings are reviewed in detail. Particularly complete are the two chapters on the gastrointestinal and cardiovascular systems. Protein metabolism and its importance in the pathogenesis of "dystrophy" and of edema are extensively discussed in a separate chapter.

The bibliography includes 361 works published up to 1949, with predominance of European and especially of German works.

This book should be of considerable interest to the student of nutritional diseases and to those physicians directly concerned with nutritional surveys and with the health of the population in countries where malnutrition is endemic.

PRÉCIS DE PARASITOLOGIE. By E. Brumpt, member of the Academy of Medicine of Paris, honorary professor of the Faculty of Medicine of Paris and honorary director of the School of Malariology of the University of Paris. Sixth edition. Two volumes. Pp. 2138, with 1305 illustrations including 4 plates, 2 in color. (Collection de précis médicaux). Price 7,700 francs. Masson & Cie, 120 Boulevard Saint-Germain, Paris-6e, 1949.

This well known and classic two volume set on parasitology and mycology has been extensively revised in its sixth edition. A massive amount of new information which was added to this field prior to and during the second World War has in part been incorporated within the text and in part included as an appendix of the second volume in order to bring the work up to date.

The format of the volumes remains basically the same. There are, however, 220 new illustrations. Not only is there a comprehensive discussion of the etiologic agent, the geographic distribution and the clinical course of each of the parasitic diseases of man but, of special interest to the pathologist, an ample morphologic description of the lesions induced by the agent.

For both the clinical pathologist and the morbid-anatomist, these volumes are indispensable. There are few textbooks that cover this important field so comprehensively and thoroughly.

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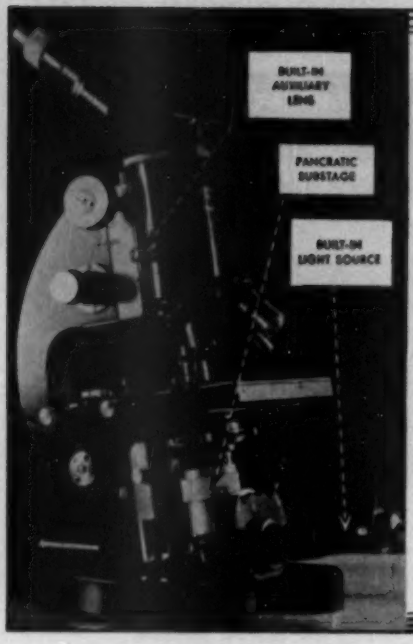

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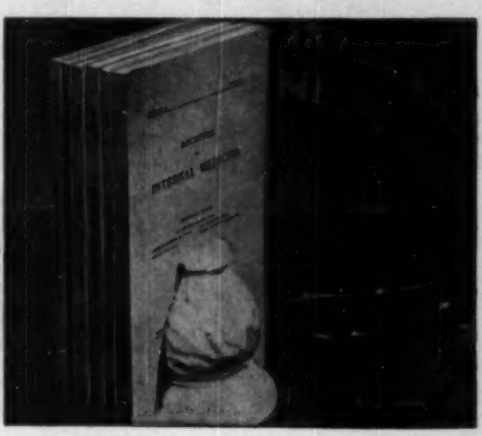

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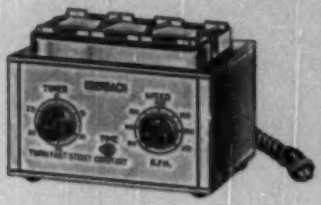

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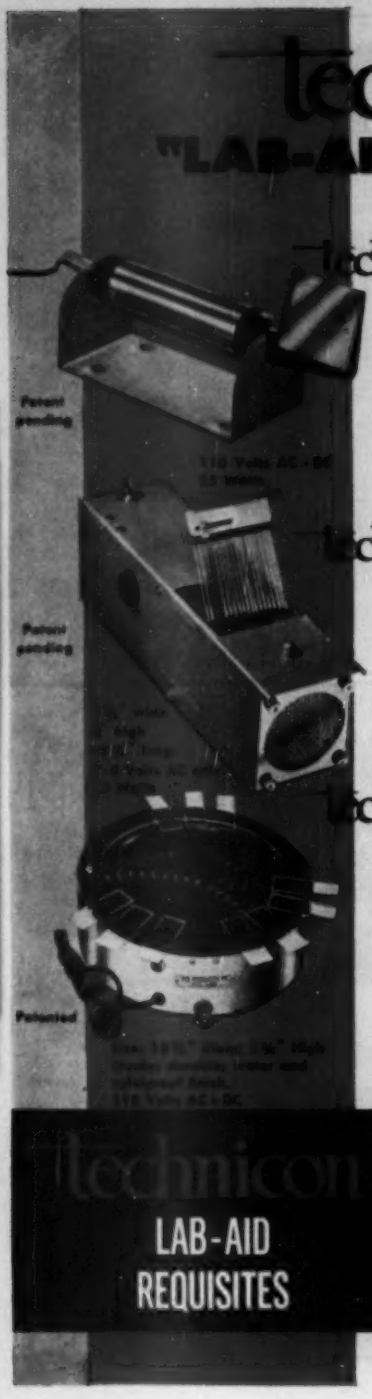

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